IMPROVING THE PERFORMANCE OF WEANER PIGS THROUGH DEVELOPMENTS IN LIQUID FEEDING

by

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ABSTRACT

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IMPROVING THE PERFORMANCE OF WEANER PIGS THROUGH DEVELOPMENTS IN LIQUID FEEDING

A programme of work was undertaken to assess the efficacy of a new automated *ad libitum* feed delivery system for newly weaned pigs; to investigate the effects of liquid feeding on their performance and to explore the possibilities for reducing diet cost by using lower cost liquid components.

A series of 28 day feeding trials was conducted using pigs weaned at 24 ± 4 days and fed *ad libitum* on liquid diets. Compared with pigs fed dry diets, liquid feeding increased feed intake by 109 ± 10 g d^{-1} ($P<0.001$) and daily gain by 57 ± 14 g d^{-1} ($P<0.001$). Pig growth and feed conversion ratio was not significantly influenced by dry matter content over the range of 255 - 149 g DM kg^{-1}. However, diets containing less than 220 g DM kg^{-1} increased effluent output per kg of liveweight gain. Within the liquid feed system a natural lactic acid fermentation occurred which reduced diet pH ≤ 4.0 and inhibited the growth of coliform bacteria. Pigs fed diets in which pH was reduced to ≤ 4.0 by acidification with either lactic acid or *Pediococcus acidilactici* had daily gains of 496 and 474 ± 17 g d^{-1} and feed conversion ratios of 1.11 and 1.15 ± 0.06 respectively.

A series of laboratory studies was conducted with the aim of upgrading and controlling fermentation of food industry liquid residues for use in liquid diets for weaners. Steeping was investigated as a method for reducing glycoalkaloid levels in reject raw potatoes. A combination of natural fermentation and hydrolysis reduced the levels of α-solanine by 16.6 mg kg^{-1} (35%) and α-chaconine by 28.7 mg kg^{-1} (51%) respectively. Diets based on the food industry liquid residues (Whey, 'C'-Starch and Greenwich Gold), were either allowed to ferment naturally or inoculated with *Enterococcus faecium* or *Pediococcus acidilactici*. Inoculation with either *Enterococcus faecium* or *Pediococcus acidilactici* did not result in a significant difference in the final pH of the diets or in the final populations of microorganisms examined compared with the control.

The series of studies demonstrated the potential for improving weaner pigs performance using fermented liquid diets. However, it highlighted the need for further studies to obtain a greater degree of control over fermentation patterns.

FREQUENTLY USED ABBREVIATIONS

ADFI	Average daily feed intake
ADLG	Average daily liveweight gain
CNS	Central nervous system
DE	Digestible energy
DM	Dry matter
DF	Dry fed
DMFCR	Dry matter feed conversion ratio
DMFI	Dry matter feed intake
DM149	Dry matter content of 149 g kg^{-1}
DM179	Dry matter content of 179 g kg^{-1}
DM224	Dry matter content of 224 g kg^{-1}
DM255	Dry matter content of 255 g kg^{-1}
EF	*Enterococcus faecium*
FCR	Feed conversion ratio
FI	Feed intake
FILR	Food industry liquid residues
GA	Glucose agar
GI	Gastrointestinal tract
HPLC	High Performance Liquid Chromatography
IG	Immunoglobulin
LA	Lactic acid
LCT	Lower critical temperature
LF	Liquid fed
MCC	MacConkey agar
ME	Metabolisable energy
MEW	Medicated Early Weaning
MJ	Megajoule
MRS	De Mann, Rogosa and Sharpe, agar
PA	*Pediococcus acidilactici*
PAMI	Passive anti-body mediated immunity
PCA	Plate count agar
PSP	Potato Steamed Peelings
PWD	Post-weaning diarrhoea
PWW	Post-weaning weight
RBCA	Rose Bengal Chloramphenicol agar
SEW	Segregated Early Weaning
TGA	Total glycoalkaloids
UCT	Upper critical temperature
VFI	Voluntary food intake
WA	Weaning age
WT	Weaning weight

GLOSSARY

Ad libitum feeding	The system in which the feed supply is unrestricted at all times.
Aerobe	An organism which can live and grow only in the presence of free oxygen.
Anaerobe	An organism which can grow in the absence or near absence of oxygen.
Bacteriocin	Compound produced by bacteria which are antagonistic against other bacteria.
Biosecurity	Elimination of pathogenic and food spoilage microorganisms
Creep Feed	Feed given to suckling pigs behind a barrier (or "creep") which allows them access to the feed but excludes the sow.
Digestible energy (DE)	The gross energy (or heat of combustion) of a feed minus the gross energy of the corresponding faeces, expressed as KJ total feed.
Facultative anaerobes	Organisms which can utilize free oxygen.
Feed conversion ratio(FCR)	The total weight of feed eaten over a period of time divided by the live-weight gain over that period.
Finishing pig	See **Growing pig.**
Gnotobiotic	Piglet borne into, and reared in sterile conditions.
Gram negative	Those bacteria which fail to stain the gram's reaction. The reaction depends on the complexity of the cell wall and has for long determined a major division between bacterial species, (appearance Pink).
Gram positive	The comparative simplicity of the cell-wall of some bacterial species allows them to be stained by Gram's procedure, (appearance Blue/Violet).
Growing pig	The term used to describe an animal at any stage of life between weaning and slaughter.
Inoculant	A live bacterial supplement, or starter culture
Immunoglobulin	A family of proteins all of which have a similar basic structure.
Isolates	To establish a pure culture of a microorganisms.

Lean tissue	The skeletal muscle of the carcass, excluding that of the head, hocks and *m. panniculus*, with all visible subcutaneous and intermuscular fatty tissue removed by dissection.
Maintenance	At the maintenance level of feeding, the requirements of the animal for nutrients for the continuity of vital processes within the body, including the replacement of obligatory losses in faeces and urine and from the skin, are just met so that the net gain or loss of nutrients and other tissue substances by the animal as a whole is zero.
Metabolisable energy (ME)	The digestible energy (DE) of a unit weight of feed less the heats of combustion of the corresponding urine and gaseous products of digestion. With pigs the gaseous products are usually considered to be insignificant and are ignored. At the maintenance level of feeding, ME, by definition, equals the daily heat production.
Obligate anaerobes	Organisms which are poisoned by free oxygen (gaseous or dissolved).
Parity	The condition or fact of having borne offspring; commonly used when describing the number of times this condition has occurred in an individual female - thus first parity, second parity and so on.
Pathogen	An organism, *e.g.* bacterium which causes disease.
Performance	As applied to growing pigs, is used to describe collectively their growth rate and feed conversion ratio.
Prophylactic	Protecting against disease, preventive.
Protein (crude)	Estimated protein content derived from chemically determined total nitrogen content. Since may proteins of animal origin have historically been found to contain c. 16% nitrogen by weight, **crude protein** is usually defined as total nitrogen x 6.25.
Suckling pig	A pig receiving milk from its dam.
Weaner pig	A newly weaned piglet.

Sources of definition Walker, (1991); Agricultural Research Council (1981); Procter, (1982).

CONTENTS

LIST OF TABLES

Chapter 1

Chapter 4

Chapter 5

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

Tables

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AUTHOR'S DECLARATION

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CHAPTER 1

LITERATURE REVIEW

1.1 Introduction

In the wild the weaning of piglets is a gradual process of adaptation from sow's milk to solid food which is normally completed 17 - 18 weeks post-partum (Jensen and Recen 1989). As natural weaning takes a long time the piglets are able to explore new feed sources in small quantities and develop their own immunity and physiological maturity whilst still receiving the nutritional and immunological support from the sow (English, Fowler, Baxton and Smith 1988).

In the normal commercial practice of pig production weaning is an abrupt process which is artificially shortened to anything from 19 to 28 days (after birth) in the UK (Partridge and Gill 1993), and as young as 14 days (after birth) in the USA. Piglets are also expected to adapt from consuming a liquid diet of sow's milk (Bolduan, Jung, Schnabel and Schneider 1988), delivered at regular intervals (Fraser 1980) and containing important immunoglobulins (Gaskins and Kelley 1995), to a diet which is dry, in meal or pellet form, and mainly cereal based (Bolduan *et al.* 1988; Close 1993). As well as the dietary changes, piglets are also subjected to mixing with other piglets, accommodation changes and separation from the sow. At this stage the piglets may be underweight, and too physiologically and immunologically immature to deal with this abrupt transition satisfactorily (Blecha, Pollman, and Nichols 1983; English *et al.* 1988; Partridge and Gill 1993; Aumaitre, Peiniau, and Madec 1995). When piglets are weaned they are usually offered a separate water and dry food supply which can create two immediate problems for the piglets. Firstly, the piglet may not locate the water supply for many hours which can lead to dehydration and possible death (Partridge and Gill 1993). Secondly, the piglet may

not recognise that dry pellets are meant to replace sow milk. When it does it may gorge itself with dry feed. As the piglet lacks the enzyme capacity (Whittemore 1993; Aumaitre *et al.* 1995) and stomach acidity (Cranwell 1995) necessary to digest dry feed the result is a sudden loading of partially undigested material of a high pH reaching the intestines where it provides a substrate for enteropathogenic bacteria (Kidder 1982; Bolduan *et al.* 1988; Hill and Sainsbury 1995). These management challenges, imposed upon the young piglet for purely economic and practical reasons, combine to produce stresses which often result in post weaning growth check, enteric disorders, and increased mortality.

The combination of weaning problems often makes it difficult to manage the piglets in a way which will allow them to reach their maximum growth potential. Management target performances are calculated on the basis of pre-weaning growth rates, with piglets being expected to attain a growth rate of 200 - 300 g d^{-1} in the first week post weaning and 500 g d^{-1} at 8 weeks of age (Close 1993). In order to attain realistic performance targets managers have used dry diets which are formulated with highly digestible materials, acidified to prevent digestive upsets, and offered very fresh usually in pellet form. Although all of these factors make the management task of feeding dry food easier and convenient it can mean that weaner dry diets are very expensive and may not provide the piglet with it's diet in the form which is most beneficial to the pig.

A viable alternative to dry feeding weaners is the use of liquid feed (Braude, 1972; Braude, 1990; Gill, 1989; Partridge and Gill 1993). It is generally accepted that feed intake, growth rate, feed conversion efficiency and health is improved when pigs are fed on liquid diets as opposed to those fed on an equivalent dry feed (Braude and Rowell, 1966; Braude, 1972; Braude and Newport 1977; Lecce, 1975). Piglets will readily consume liquid diets, and feeding weaner pigs on liquid diets has been attempted on a number of occasions (Braude,

1972; Kornegay and Thomas 1981; Partridge and Gill 1993; Upton, 1993). The major problem has been to devise suitable equipment and maintain the feed in a hygienic and palatable state (English *et al.* 1988). Currently, new systems which are computer controlled and provide more accurate delivery than dry feed systems have been developed which allow greater flexibility in raw material use, and are less labour intensive.

Research by other workers has shown that there are many advantages to be gained from feeding liquid diets to pigs. For example, reduction in food lost through dust can be minimised (Standing Committee on Agriculture 1987; Robertson 1994) as well as respiratory problems for both pigs and livestock personnel (Hill and Sainsbury 1995). Liquid feeding systems also offer greater flexibility in raw material usage for example facilitating the incorporation of cheaper food industry liquid residues (Gill, 1989; Cumby, 1986; De-Boer, 1983). There exists the potential in the UK to reduce feed cost per kg gain by up to £5.91 per pig if food industry liquid residues are included in pig diets (MLC 1994). A greater proportion of pigs are fed on food industry liquid residues in European countries than in UK. There is no reliable estimate of the number of pigs in the UK fed on food industry liquid residues (Brooks and McGill 1995).

Liquid feed systems can be enhanced further by the use of inoculants to control fermentation. Inoculants, which are essentially live bacterial supplements, can be used to modify the gut microflora (Fuller and Cole 1989; Fuller 1992a; Chesson 1994; Ewing and Cole 1994), and recently there has been a great deal of interest in using inoculants as an alternative to antibiotics in pig diets. However, to date these have been used mainly in dry diets with variable results (Pollman 1986). Uncontrolled fermentation of liquid diets for pigs, especially where food industry liquid residues are used, can give rise to undesirable yeast fermentations and contamination by pathogenic organisms. Therefore, a greater

degree of control over fermentation is essential if liquid feed systems are to be operated safely. Lactic acid fermentation is widely used in the human food processing industry where inoculum recycling is employed (Dillon and Cook 1994). This process can be easily adapted to liquid feed systems for newly weaned piglets to give a greater degree of biosecurity (Urlings, Mul, Klooster, Bijker, Logtestijn and Gils 1993). Biosecurity may arise as a result of stabilisation of the liquid and protection against pathogens *via* the production of antimicrobial metabolites, which include organic acids, alcohols, and bacteriocins (Dillon and Cook 1994).

1.2 Liquid feeding of pigs

The practice of liquid feeding weaner pigs goes back to the 19th century (and probably beyond that). Both (Henderson 1814) and (Youatt 1847) considered that newly weaned pigs required frequent small meals mixed with water and fed as warm as sow's milk. In the early part of this century pigs were commonly fed their meal soaked with water before feeding (King 1982). In the last quarter of a century there has been a renewed interest in liquid feeding pigs (Lawrence 1982). Braude, (1972) reviewed 61 studies (published between 1956 and 1972) comparing pigs fed on liquid or dry diets . He concluded that:-

> 'the majority of reports favour wet feeding; a considerable number of reports found no difference between wet and dry feeding and none points to the superiority of dry feeding'.

Since Braude, (1972) published his review on liquid feeding there have been very few studies published which have compared the effects of feeding liquid or dry diets. The majority of these are presented in (Table 1.1). It would appear from this analysis that little has changed since Braude's 1972 review.

Table 1.1 Review of papers published comparing the effects of liquid *versus* dry feeding for pigs.

Growth rate	Feed-gain ratio	Carcass quality	No of reports	References
+	+	0	1	(Kneale 1971)
+	+	0	1	(Smith 1976)
+	+	N	1	(Partridge, Fisher, Gregory and Prior 1992)
+	+	N	1	(Upton 1993)
+	+	N	2	(Pluske, Williams and Aherne 1996a) (Pluske, Williams and Aherne 1996b)
+	+	-	1	(Patterson 1989b)
+	0	N	1	(Braude and Newport 1977)
+	N	N	1	(Lecce, Armstrong, Crawford and Ducharme 1979)
0	-	N	1	(Kornegay and Thomas 1981)

+, liquid better; - dry better; 0 no difference; N, no information

The extent to which liquid diets are used varies greatly between countries. In the UK around 25% of growing and finishing pigs are fed liquid diets (P. McTiffin personal communication 1996). In the USA the system is rarely used while in some East European countries 50% or more of pigs may be fed liquid diets (Brooks and McGill 1995). Although liquid feeding has been shown to be beneficial in older pigs (Gill, Brooks and Carpenter 1987; Barber, Brooks and Carpenter 1991) it has not been widely used for newly weaned piglets. This is primarily due to the practical difficulties of maintaining feed hygiene (English *et al.* 1988); the increased labour requirements for equipment cleaning and refilling the feed system (Partridge and Gill 1993) and the very low dry matter content of the diet needed to optimise delivery limiting dry matter intake and reducing performance (English *et al.* 1988). As the majority of farms are not equipped to feed newly weaned piglets on liquid diets there would need to be compelling reasons for them to consider doing so. Cumby (1986) considered that since 1976 there has been a significant increase in the number of pigs fed liquid diets. He suggested that the increased popularity of liquid feeding is mainly due to the improved efficiency and/or reduced cost which they are said

to provide. Other researchers (Braude 1972; Lawrence 1982; Patterson 1989a) considered that liquid feeding reduced wastage and that this was a very important advantage over dry feeding. Partridge and Gill (1993) suggested that liquid feed was advantageous for weaner pigs as they did not have to learn separate patterns of feeding and drinking behaviour thereby avoiding the deleterious affects of dehydration. Lawrence (1982) stated that;

> 'The general thesis is that soaking improves the nutritive value of the diet by its action on the cereal fraction, damaged cereal starch being converted to dextrin and reducing sugars by either natural cereal enzymes action and/or by enzymes produced by the growth of microorganisms'

Subsequently, Barber (1992) demonstrated that dry matter digestibility of diets was increased by liquid feeding the pig and there was some evidence that nitrogen retention was also improved.

For the purposes of the subsequent review it is necessary to produce a working definition of liquid feeding.

Definition of liquid feeding

Liquid feeding involves the use of a diet prepared either from liquid components or from dry components, thoroughly mixed with water at a central point and subsequently transported to the pig. Typically a liquid diet will contain 200 - 300 g dry matter kg^{-1}.

As this review is concerned with newly weaned pigs, whose genetic potential has changed over the last 15 years and whose minimum weaning age must exceed 21 days (OJEC 1991), papers have only been included if they satisfy the following criteria:

1) the experiments had been reported since 1980 and included data on the effects of liquid feeding on growth performance

2) the weaner pigs used in the experiments were aged 21 days or more and weighed not more than 20 kg

If the definition of liquid feeding above did not apply then the conditions of the experiment have been described. A review of the literature suggests that liquid feeding of newly weaned piglets has many advantages and few disadvantages (Table 1.2).

Table 1.2 Some advantages and disadvantages of using liquid feed (LF) systems for newly weaned piglets

Advantages	Disadvantages
LF is effective in maximising nutrient intake[a]	Initial cost of LF systems may be prohibitive[b]
LF allows the substitution of cheaper food industry liquid residues[c]	Uncontrolled fermentation can occur in LF systems[d]
LF can reduce dust in piggeries which will improve health of pigs and stockmen[e]	Carcass quality may be affected[f]
LF can be used to recycle potentially polluting food industry liquid residues[g]	Noise levels of the LF operating systems may cause ear damage[h]
Performance can be improved, and gut physiology maintained[i]	LF left in troughs can sour quickly[j]

[a] (Upton 1993; Whittemore 1993; Pluske *et al.* 1996a; Pluske *et al.* 1996b);
[b] (Cumby 1986; Upton 1993);
[c] (De-Boer 1983; Cumby 1986; MAFF 1986; Gill 1989; MLC 1994);
[d] (P. McTiffin, personal communication 1996);
[e] (Cumby 1986; Robertson 1994; Hill and Sainsbury 1995);
[f] (Patterson 1989b);
[g] (Perry 1995);
[h] (Cumby 1986);
[i] (Upton 1993; Pluske *et al.* 1996a; Pluske *et al.* 1996b);
[j] (English *et al.* 1988).

It has to be recognised that since Braude (1972) conducted his review on liquid feeding, pig performance has changed dramatically. In the last 15 years the genetic potential of the pig has changed considerably resulting in an animal with higher lean growth potential and improved feed conversion efficiency (Webb 1989). These new improved genotypes have enormous genetic potential for growth which as yet has not been fully exploited (Bolduan *et al.* 1988; Pluske, Williams and Aherne 1995).

Liquid feed systems are flexible enough to permit the use of a wide range of human food industry liquid residues (FILR) such as whey, skimmed milk, distillery waste, liquified fish, grain processing wastes, sugar industry wastes, potato processing wastes and many others (Brooks and McGill 1995). In the Netherlands large quantities of FILR are fed to pigs (Table 1.3).

Table 1.3 Annual consumption of food industry liquid residues used in liquid feeding systems by pigs in the Netherlands

FILR	Quantity fed to pigs (x 1000 kg y^{-1})	Dry matter (g kg^{-1})
Grain processing industry		
wheat starch	835,000	20
brewer's yeast	75,000	14
spent grain	25,000	10
alcohol stillage	500	12
Potato processing industry		
potato steamed peelings	250,000	14
Dairy industry		
whey	250,000	3
whey concentrates	45,000	5
Fruit and vegetable processing industry		
onion juice	50,000	
mustard seed	30,000	
carrot steamed peelings	2,000	10
Soya processing industry		
soya whey concentrate	20,000	

(Go 1996)

There are distinct economic advantages for using FILR in liquid feed systems for pigs compared to purchasing dry compound weaner diets. Data from the Meat and Livestock Commission (MLC) Feed Recording Scheme 1993 indicated that the average feed cost per tonne for feeding herds using compound feeds was £177.94. For herds where home mixed feeds were used, cost per tonne was reduced to £155.84, and those producers using co-products had a feed cost per tonne of only £67.02. As a consequence of this, feed cost per kg gain was respectively 45.88, 41.24 and 37.43 pence (MLC 1994). For a pig growing from 20 to 90 kg this would represent a saving of £5.91. In the Nord-Pas de Calais region of France the use of FILR has been reported to reduce the cost of pig feed by 35% compared to an ordinary pig feed purchased locally (Jenkins 1994).

In the UK liquid feeding newly weaned pigs is a relatively new concept but with the invention of new, fully automatic, liquid feeding systems, it can be very successful and cost effective. For example, one Dorset producer currently feeds piglets from 10 kg liveweight on to a liquid diet consisting of home grown cereals, whey, cider making waste, and Abrapro (residue after wet milling cereals). For pigs weighing between 20 and 65 kg this resulted in a feed cost of 26 pence kg^{-1} gain (Jordan 1995). This figure is even lower than the MLC figure of 37.43 pence kg^{-1} for pigs fed on co-product diets and gives an indication of the magnitude of savings which are possible.

Efird, Armstrong and Herman (1982b) compared the effects of feeding liquid or dry diets containing 24% milk protein to piglets weaned at 21 days. They demonstrated that the liquid fed pigs had better feed to gain ratios (1.11) compared with 1.82 for the dry fed pigs. In a later experiment Partridge *et al.* (1992) demonstrated that pigs fed liquid diets had significantly higher feed intakes ($P<0.05$) and as a result had significantly higher ($P<0.01$) growth rates (312 *vs* 281 g d^{-1} respectively) than dry fed pigs. In the experiment of

Partridge *et al.* (1992) the liquid feed system used a device called Autofeeder which dispensed a compound pelleted diet plus water (1:1 ratio) 6, 8, 12 or 24 times throughout the day in varying quantities.

The most recent experiments reported which have examined the effects of liquid feeding on newly weaned piglets have been conducted in Australia (Pluske *et al.* 1996a; Pluske *et al.* 1996b). They demonstrated that piglets, weaned at 28 days and fed *ad libitum* on liquid diets grew significantly ($P<0.001$) better in the 5 days after weaning and consumed more dry matter (400 *vs* 286 g d^{-1}, $P<0.010$) than pigs fed a dry starter diet. They demonstrated the interdependence between voluntary food intake and mucosal architecture in determining piglet performance after weaning.

To explain how and why liquid feeding confers benefits upon the young pig it is necessary to examine the effect of diet form on the physiological development of the young pig. This is the subject of the following sections.

1.3 Immunological development of the piglet

The piglet is born with a low immunocompetency because the sow possesses a specialized epitheliochorial placentation which does not permit the passage of maternal antibodies (immunoglobulins, Ig) to the foetus (Pond and Houpt 1978; Gaskins and Kelley 1995). Immunoglobulins are structurally related proteins which function as anti-bodies (Procter 1982). When the piglet is born its immune system is also anatomically and functionally immature (Gaskins 1996). This means that immediately after birth the piglet is dependent on acquiring immunity in the form of maternal antibodies which are transferred *via* sow's milk. This type of immunity is referred to as passive anti-body mediated immunity (PAMI) (Blecha *et al.* 1983). Acquiring PAMI is essential if the neonatal piglet is to resist

disease and be protected against many of the specific potential diseases associated with any particular herd (English *et al.* 1988). The maximum Ig absorption in the neonatal piglet occurs within 4 - 12 hours after suckling and then rapidly declines due to a gradual and progressive process known as gut closure (Pond and Houpt 1978; Gaskins 1996). During this period colostral Igs can cross the gut wall and enter the bloodstream. When gut closure is complete direct absorbtion is no longer possible (Pond and Houpt 1978; Gaskins 1996). PAMI reaches a maximum in the piglet when 24 - 36 hours old, thereafter decreasing to precariously low levels when about 3 weeks old (Blecha *et al.* 1983).

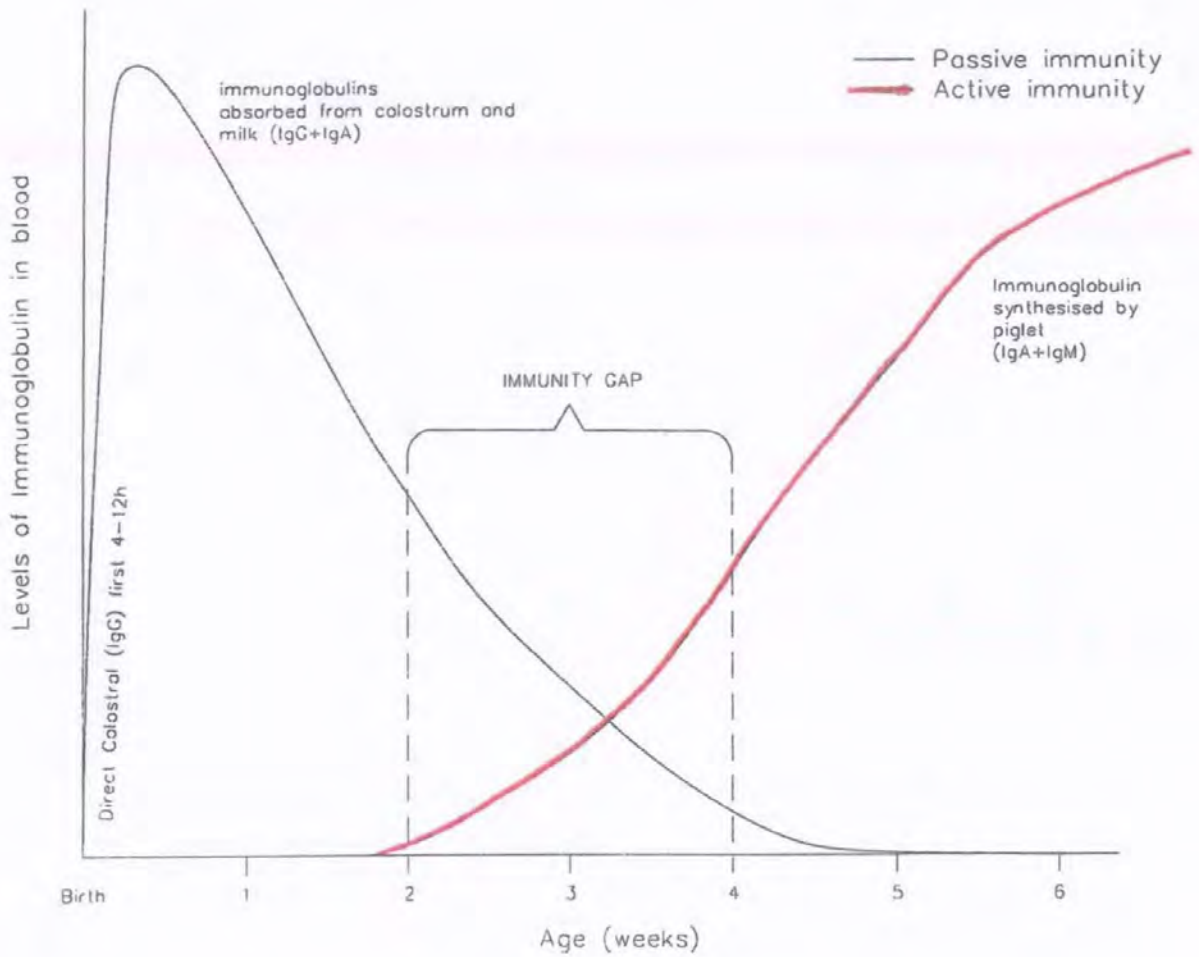
There are several types of Igs, the main three being IgG, IgA and IgM, and the piglet needs a minimum amount of all of them (English *et al.* 1988). The type of Ig initially received by the piglet is IgG, and depends on previous exposure of the sow to antigens and her ability to develop memory B cells. As the formation of colostrum declines and lactation proceeds the IgG concentrations decline and are replaced by IgA from the sows milk. The concentrations of Ig's present in the sows milk are in the approximate proportions of 20 - 30% for IgG, 50 - 60% for IgA and 18% for IgM (Pond and Houpt 1978). IgA provides the piglet with protection in the small intestine by suppressing the effect of some disease-causing viruses such as corona virus and rotavirus (English *et al.* 1988). Sow's milk also contains other defence factors such as lactoferrin and lysozyme. The PAMI which is acquired from the sow's milk, protects the piglet for the first 3 - 4 weeks of age whilst it develops its own 'active immunity' (McCracken and Kelly 1993). Since the piglet does not synthesise antibodies very well its immunity remains low until 6 - 8 weeks of age (Cromwell 1991). Active immunity develops as a result of the piglet being able to make copies of the sow's antibodies (English *et al.* 1988) and also as a result of exposure to pathogenic organisms in its environment (Gaskins and Kelley 1995).

Since passive immunity only lasts for the first few weeks and active immunity is not fully developed for at least 6 weeks, weaning at less than 2 weeks of age poses a serious challenge to the piglets ability to combat disease immediately post weaning. The period between 2 and 3 weeks of age is sometimes referred to as the immunity gap (Figure 1.1). Weaning later than 3 to 4 weeks of age improves the piglet's chances of fighting disease as the piglet will have a better developed immune system (English *et al.* 1988). Blecha *et al.* (1983) weaned pigs at 2, 3, 4 or 5 weeks of age to assess the affects of weaning on cellular immunity. Their data suggested that a weaning age of < 3 weeks would compromise the immune status of the piglets. Weaning pigs < 5 weeks of age caused physiological changes detrimental to cellular immune reaction which could alter disease susceptibility. Close (1993) has stated that,

> "It is not known whether these immuno-suppressive actions are caused by the change at weaning from a liquid, milk based diet to a solid cereal based diet or by other stresses that the piglet is subjected to at this time".

When an animal is exposed to substance foreign to its body it's immune system is activated. These substances (antigens) include viruses, bacteria and soya proteins (Stahly 1996). The newly weaned piglet is likely to encounter some of these substances from its diet and surroundings. The response of an animal's immune system to these antigens will be the release of a series of compounds called cytokines. These in turn activate the cellular and humoral components of the immune system. Mounting an immune response is costly in terms of energy to the animal and can reduce growth performance. In a recent review Stahly (1996) presented data which demonstrated that chronic activation of the immune system of pigs weighing 6 to 27 kg resulted in poorer growth performance compared to pigs with low immune system activation (Table 1.4).

Figure 1.1 The immunological development of the newborn piglet.



Constructed from information given by Gaskins 1996; Cromwell 1991; McCracken and Kelly 1993; and English *et al.* 1988.

Table 1.4 Impact of level of chronic immune system (IS) activation on growth performance in pigs fed from 6 to 27 kilograms bodyweight.

Item	IS activation	
	Low	High
Pig body weight, kg		
Initial	6.4	5.9
Final	27.2	25.9
Growth and feed utilization		
Daily feed, g	973	863
Daily gain, g	676	477
Feed/gain	1.44	1.81

Adapted from Stahly (1996) data from (Williams, Stahly, Zimmerman and Wannemuehler 1993b; Williams, Stahly and Zimmerman 1993a).

It is known that weaning stress causes significant changes in some components of the immune system (Svendsen and Svendsen 1987). Metz and Gonyou (1990) examined haemolytic reactions as a parameter of physiological stress in early weaned pigs, and reported that the stress response to weaning was greater in 2 week old piglets than 4 week old piglets. Svendsen and Svendsen (1987) suggested that the degree of change in the immune system depends on the type of stressor (for example intermittent draughts or inappropriate temperatures post weaning) and duration of exposure. Therefore, the environment and diet of immunocompromised newly weaned piglets needs careful management if they are to thrive.

In order to overcome the effects of reduced immunity and the consequences of disease susceptibility whilst still exploiting the growth potential of the newly weaned pig some producers have adopted systems such as medicated early weaning (MEW) (Pluske *et al.* 1995) and segregated early weaning (SEW). The concept of MEW involves supplementing the passive protection of the piglets. Sows are given prophylactic medication from the time of entering the isolated farrowing unit to the time of weaning, combined with vaccination

1 - 2 weeks before farrowing (Hill and Sainsbury 1995). The piglets are given antibiotics from birth until weaning to reduce any disease risk. The theory is that it is possible to obtain 'clean' piglets from a 'dirty' sow herd and avoid the immunity gap by weaning before it.

The concept of SEW is that piglets are removed from the sow whilst they are still protected with maternal antibiodies and in theory are disease free. They are then taken to a clean site where they will be free from a disease challenge. It has been recognised for years that an all-in/all-out stocking system works well for the health of pigs but SEW depends heavily on the ability of the stockpersons to maintain an almost sterile environment and has to be backed up with antibiotic treatment (Best 1996). Caldwell (1996) suggests some of the achievements due to SEW are a decrease in many conventional diseases which in turn allows for genetic potential performance to be expressed in a larger percentage of pigs and a decreased cost of disease.

Caldwell (1996) has commented that MEW may be compromised by multi-parity sows and weaning piglets of different ages. It is the mature second or third parity sows in a microbiologically stable herd which become immune to most of the more virulent infectious pathogens endemic in the herd and cease to shed or carry them (Hill and Sainsbury 1995). Young sows may not have acquired the same level of immunity as second or third parity sows. Caldwell (1996) has pointed out that there are several factors which may lead to a potential breakdown of SEW which may result in subsequent disease outbreaks in piglets. He suggests that these factors are: non-immune females, sick sows not milking properly, an over concentration of pathogens, uncontrolled weaning ages with back fostering resulting in older piglets transferring disease to younger piglets.

It has also been suggested that transient hypersensitivity to food antigens in the immediate post weaning period of piglets can evoke an immune response which may cause damage to the small intestinal surface and predispose weaned piglets to an *Escherichia coli* enteritis (Miller, Newby, Stokes and Bourne 1984a; Miller, Newby, Stokes and Bourne 1984b; Newby, Miller, Stokes, Hampson and Bourne 1985). The antigens contained in soya-proteins have been particularly implicated (Newby *et al.* 1985). Li, Nelssen, Reddy, Blecha, Klemm and Goddband (1991b) 'sensitised' young pigs prior to weaning with dried skim milk, or a variety of soya products. They found that the antigens in soya (in particular glycinin and beta conglycinin) evoked an immune response which was shown to be detrimental to post weaning growth rates. In their study milk based proteins maintained a superior gut structure when compared with all soya products. Interestingly, the percentage of coliform bacteria was higher in those piglets exposed to untreated soya proteins (Table 1.5).

Table 1.5 The effect of different soya products on the immune response and post weaning growth rates of young pigs aged 21 days

	Milk protein	Soyabean meal	Soya protein concentrate 1	Extruded soya protein concentrate
Residual antigens in products[a]				
Glycinin	-	2.4	0.9	0.8
β-Conglycinin	-	3.6	1.5	1.3
Villus height (μm)[bc]	364	234	309	319
Crypt depth (μm)[cd]	198	222	214	196
Coliforms				
(% total bacteria)[b]	1.7	37.0	24.3	4.0
ADG,g				
(0-14 d)[e]	326	182	208	227
DMFI,g				
(0-14 d)[f]	301	251	231	243

[a] Enzyme-linked immunosorbent assay titres (log_2);
[b] Milk protein differed from others (P<0.01):
[c] Soybean meal differed from mean of soy products (P<0.1):
[d] Milk protein differed from soy bean meal (P<0.01):
[e] Milk protein differed from soybean meal (P<0.5).
[f] Soy bean meal differed from other soy products (P<0.01)
Adapted from (Li, *et al.* 1991b).

Hall and Byrne (1980) examined the effect of dietary changes on small intestinal structure and function in gnotobiotic piglets and found that antibodies to soya antigens were detected in serum after weaning. They suggested that intestinal damage could have been caused by antibody mediated immune reactions to soya proteins. The use of gnotobiotic pigs enabled Hall and Byrne (1980) to study a direct effect of dietary change, on the structure and function of the small intestine uncomplicated by interactions with microbial populations. From their studies, they suggested that there was no evidence that creep feeding provoked an immune response. They observed that the most severe gut damage occurred in those piglets subjected to an abrupt dietary change and not those which had experienced a sensitising exposure to the antigens in the soya diet. There does appear to be some debate as to whether the use of creep feed, which contains antigenic compounds, sensitises piglets post weaning (Partridge and Gill 1993), because Hampson, Fu and Smith (1988) and Kelly, Smyth and McCracken (1990) failed to reproduce a hypersensitivity response in post weaned piglets which had previously been exposed to creep food.

Current legislation prohibits the inclusion of meat and bone meal in animal feeds in the UK, thereby increasing the demand for vegetable protein, such as soyabean. However, if soyabean is to be used successfully in weaner diets then it needs to be treated to remove antigenic compounds in order to prevent immune responses and gut damage. Partridge and Gill (1993) reviewed the importance of feed processing to reduce the effects of antigens in soya protein destined for weaner diets. There appears to be some benefit from extrusion processing (*i.e.* subjecting material to high temperature, pressure and friction), as this denatures the protein, making it more susceptible to enzymic breakdown and also kills pathogens.

1.4 Development of the microflora of the piglet

The gastrointestinal (GI) tract of the newly born pig is bacteriologically sterile (Kenworthy and Crabb 1963). During the first day of the piglets life the GI tract is colonised by bacteria (Tannock 1992). Small microbial populations have been detected in animals within 3 h of birth (Smith and Jones 1963; Sinkovics and Juhasz 1974; Savage 1977). Within 48 hours the gastric contents of the piglet have a sufficiently low pH, *circa* 2.0 to 4.9, to prevent the multiplication of all ingested bacteria except *Lactobacilli* which continue to proliferate in the stomach and GI tract (Smith and Jones 1963; Smith 1965).

The primary source of the microorganisms which colonise the gut is the sow and the piglet's immediate surroundings (Smith 1965; Kovacs, Nagy and Sinkovics 1972; Sinkovics and Juhasz 1974; Tannock 1992; Maxwell and Stewart 1995). It has been demonstrated by Sansom and Gleed (1981) that piglets consume considerable quantities of sow's faeces and bedding (mean weight ingested 20 g d^{-1}) which provides a source of inoculation by microorganisms. Sow's faeces are known to contain a range of microorganisms which are predominantly anaerobic (Mitsuoka 1982) (Table 1.6).

Tannock, Fuller and Pedersen (1990a) demonstrated that the sow's faeces were a major source of the *Lactobacilli* colonizing the piglet's digestive system and they described these organisms as part of the 'piggery microflora' that contaminates the neonatal piglet. Naito, Hayashidani, Kaneko, Ogawa and Benno (1995) identified four species of *Lactobacillus* from sow's faeces; namely *Lactobacillus acidophilus*, *Lactobacillus salivarius*, *Lactobacillus casei* and *Lactobacillus reuteri*. Salanitro, Blake and Muirhead (1977) demonstrated that Steptococci constituted 44% of the cultivable microbes in sow's faeces.

Table 1.6 Microorganisms present in the faeces of adult pigs

Bacterial groups	Log number
Bacteroidaceae [oa]	10.3 ± 0.8 (5)
Eubacteria [oa]	9.2 ± 1.0 (5)
Peptococcaceae [a]	9.8 ± 0.3 (5)
Anaerobic curved rods [a]	9.4 ± 0.3 (5)
Bifidobacteria [a,fa]	9.0 ± 0.5 (5)
Lactobacilli [a,fa]	9.9 ± 0.4 (5)
Clostridia [a]	6.9 ± 1.0 (4)
Spirochaetes [a,fa,aa]	9.5 ± 0.8 (3)
Enterobacteriaceae [fa]	8.1 ± 0.1 (5)
Streptococci [fa]	7.9 ± 1.0 (5)
Staphylococci [fa]	3.5 ± 1.1 (3)
Corynebacteria [a,fa]	6.5 ± 0.5 (2)
Bacilli [a,aa]	6.4 ± 0.9 (5)

[a] Mean ± SD of log bacterial counts (when present);
[b] Figures in parentheses refer to the number of subjects that harbour the organism;
[oa] Obligate anaerobes;
[a] Anaerobes;
[fa] Facultative anaerobes;
[aa] Aerobes.
Adapted from (Mitsuoka 1982).

Studies conducted before 1970, which examined the colonization of the porcine GI tract, used techniques which may have under represented certain species of anaerobic bacteria (Salanitro *et al.* 1977; Savage 1977; Allison, Robinson, Bucklin and Booth 1979; Robinson, Allison and Bucklin 1981; Tannock 1983; Barnes 1986). More recently methods have evolved which use 'strictly anaerobic techniques'; initially developed by Hungate (1950), and further adapted by others (Mitsuoka 1982). These technical improvements have increased cultural recoveries from intestinal contents (Mitsuoka 1982) and should, in theory, overcome problems of under representation of anaerobic species (Salanitro *et al.* 1977; Robinson *et al.* 1981). Other studies have observed changes in the microflora of the pig using faecal samples as an indicator of the microorganisms present in the GI tract. However, Tannock *et al.* (1990a) and Pollman, Danielson and Peo (1980) have demonstrated that pig faeces may not be a good indicator of the actual microflora of the

GI tract. Therefore, in reviewing the subject of the changing development and successive colonisation of the piglet microflora, only papers satisfying the following criteria have been included:

1. experiments which utilised only 'strictly anaerobic techniques' have been referred to where the total microflora of the pigs GI tract was discussed
2. experiments which sampled directly from the contents of the GI tract, and not the faeces, were referred to when comparing changes in microflora of the pigs GI tract.

Pedersen and Tannock (1989) examined the GI tract of neonatal piglets and demonstrated that piglets were colonized by a mixture of Lactobacilli from the environment within 1 day of birth and that they were present throughout the GI tract, increasing ten fold 10 days after birth. Subsequently, Tannock *et al.* (1990a) used plasmid profiling of *Lactobacillus* isolates to observe the successive colonisation of *Lactobacillus* strains which inhabited the pars oesophageal surface during the first 7 days of the piglets life. They identified the *Lactobacillus* species as being *Lactobacillus acidophilus*, *Lactobacillus brevis*, *Lactobacillus delbreukii* and *Lactobacillus fermentum* and have demonstrated that the microflora ecosystem of the GI tract of pigs is dynamic and changes over time. This theory is in opposition to views held previously by Savage (1977), who suggested that a stable state of microflora was reached in animals when they matured.

In most animals, the initial colonizing organisms are non pathogenic strains of *Escherichia coli*, Streptococci, Clostridia and *Lactobacilli* (Barnes 1986). Later, as the oxygen is removed from the GI tract by facultative bacteria, the anaerobic bacteria become dominant (Barnes 1986). Smith and Jones (1963) demonstrated that Bacteroides were also found in

the caecum, large intestine and rectum of healthy piglets from 2 days of age. Studies have also shown that different sections of the GI tract contain different populations of microorganisms (Kenworthy and Crabb 1963; Kovacs *et al.* 1972; Allison *et al.* 1979; Russell 1979). It has also been demonstrated that the GI tract can be divided spatially into micro-habitats which harbour different populations of microorganisms. For example Russell (1979) and Kovacs *et al.* (1972) analyzed various sites along the large intestine of 20 to 25 week old pigs. These sites included the lumenal content, the lumenal surface and the intestinal wall tissue. The numbers of microorganisms found in these sites were 13.3 x 10^{10}, 14.0 x 10^{10} and 5.1 x 10^{10} (organisms per gram dry weight) respectively.

1.5 The consequences of weaning on the microflora of the Gastrointestinal tract of the piglet

All the available data support the belief that the indigenous, anaerobic microflora performs an essential role in the health and well being of the animal and acts as a protective barrier against colonisation by pathogens (Muralidhara, Sheggeby, Elliker, England and Sandine 1977; Pollman *et al.* 1980; Tannock 1990; Maxwell and Stewart 1995). This has led to the development of probiotic strategies. The subject of probiotics has been previously been reviewed (Pollman 1986; Fuller 1989; Fuller and Cole 1989; Haresign and Ewing 1989; Lyons 1989; Peitersen 1991; Fuller 1992b; Huis in't Veld and Havenaar 1993; Stewart and Chesson 1993; Chesson 1994; Maxwell and Stewart 1995; Stavric and Kornegay 1995). Parker (1974) defined probiotics as " organisms and substances which contribute to the intestinal microbial balance". Fuller (1989) re-defined the term probiotic as "a live-microbial feed supplement which beneficially affects the host animal by improving its intestinal balance". The scientific basis for the development of probiotics stems from the knowledge that the gut flora is involved in protecting the host animal against colonisation of the GI tract by non-indigenous microorganisms (Fuller 1992a). At times of stress, such

as weaning, the 'balance' of intestinal microflora may become disturbed and disorders in digestive function are likely to occur (Sissons 1989). McGillivery and Cranwell (1992) examined the microflora associated with the pars oesophageal of piglets from 1 to 35 days of age. They demonstrated that the microbial population of suckling pigs varied little from birth to weaning, and that *Lactobacillus*, *Clostridium* and *Eubacterium* were the predominant species. However, when pre-weaned piglets were compared with weaned piglets differences in the microflora were observed. Microscopic examination of gram-stained sections of tissue revealed that fewer microorganisms were associated with the pars oesophageal surface in weaned than pre-weaned piglets (Table 1.7).

Table 1.7 Total number and proportion of each genus of microorganisms adherent to the pars oesophageal of pigs pre- and post weaning.

Age (days)	pre-1	pre-4	pre-10	post-21	post-28	post-35
Viable count cm^{-2} (log_{10})	7.0	6.7	6.8	5.5	5.5	5.9
Genus (% of total count)	%	%	%	%	%	%
Eubacterium spp [oa]	24		42		55	4
Lactobacillus spp [a,fa]	76	84	42	50	42	85
Clostridium spp [a]		10	5	13		11
Bifidobacterium spp [a,fa]			10			
Staphylococcus spp [fa]			5	25		
Streptococcus spp [fa]			1	12		
Escherichia coli [fa]					3	

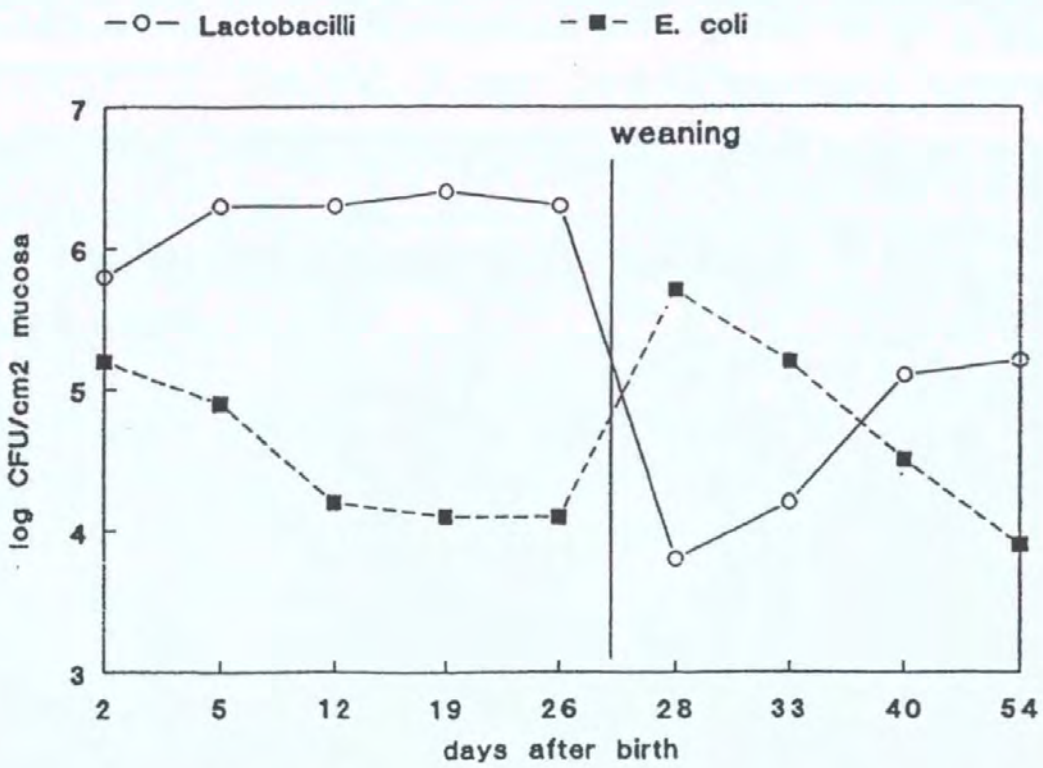
pre = pre weaned piglets;
post = post-weaned piglets.
oa Obligate anaerobes;
a Anaerobes;
fa Facultative anaerobes.
Adapted from (McGillivery and Cranwell 1992).

The intestinal bacterial flora influence the health of the host and a complicated set of interrelationships exists. These have been previously reviewed (Fuller and Briggs 1962; Savage 1977; Mitsuoka 1982; Tannock 1992). Mitsuoka (1982) considered that there were three groups of intestinal bacteria; synergistic, ubiquitous and pathogenic. He suggested that the first group were synergistic intestinal microflora benefiting the host due to their

involvement in vitamin and protein synthesis, digestion, and stimulation of the immune responses. The second group were described as ubiquitous intestinal microflora, such as *Escherichia coli* and *Streptococcus* spp. which were detrimental because they produced intestinal putrefaction, carcinogens and toxins, but were not in predominant numbers. The third group included pathogenic bacteria and were implemented in causing pathogenic diseases. Both Savage (1977) and Mitsuoka (1982) reached the conclusion that, despite accumulated knowledge of intestinal microflora and GI function, many of the operating mechanisms and factors influencing the microbial population of the gut remains unknown.

It is known that abrupt changes in the diet, starvation, water deprivation and stress can adversely affect the normal microflora of the GI tract (Mitsuoka 1982; Tannock 1983). As weaning is perhaps the major physiological and emotional stress suffered by young animals (Tannock 1983) it is not surprising that the weaning period of the piglet is often associated with disease, for example post weaning diarrhoea (PWD) (Smith and Jones 1963; McAllister, Kurtz and Short 1979; Conway, Welin and Cohen 1990; Nabuurs 1995). Hampson, Hinton and Kidder (1985) found that the number of haemolytic *Escherichia coli* and rotavirus were significantly increased in the small intestines of piglets when they were weaned. Huis in't Veld (1993) studied the development of intestinal microflora in 1 to 8 week old piglets weaned at 28 days. Their results compared counts of *Lactobacillus* and *Escherichia coli* and demonstrated that a critical point existed immediately after weaning. The numbers of lactobacilli dropped dramatically, while the numbers of *Escherichia coli* increased far above the numbers of lactobacilli (Figure 1.2). This change in microflora could lead to an overgrowth by enteropathogenic *Escherichia coli* resulting in diarrhoea.

Figure 1.2 Changes in the numbers of lactobacilli and *Escherichia coli* in the gastrointestinal tract of the piglet at weaning.



After Huis in't Veld and Havenaar (1993).

There has been a great deal of interest in examining the reasons why piglets develop PWD because it causes great economic losses due to retarded growth, cost of medical intervention and sometimes death of piglets. *Escherichia coli* strains are generally considered to be the main cause of diarrhoea at weaning (McAllister *et al.* 1979; Tzipori, Chandler, Smith, Makin and Hennessey 1980; Nabuurs, Van-Zijderveld, and De Leeuw 1993; Nabuurs 1995). Other factors may predispose the piglet to enterotoxigenic *Escherichia coli* infection. These include, hypersensitivity damage to the small intestine, viral infection, changing immunity status of the piglet and lack of sufficient digestive enzymes (Nabuurs 1995). This was in agreement with Lecce, Basbaugh, Clare and King (1982) who found that rotavirus infection damaged the small intestine of the weaned piglets which changed the luminal environment to favour of colonisation by enteropathogenic *Escherichia coli.* Kovacs *et al.* (1972) concluded that environmental conditions had a demonstrable influence on the bacteriological status of the GI tract of piglets. Farm reared piglets carried a significantly larger coliform population in both the small and large intestine than their age-mates reared in climatic chambers.

Many pathogenic bacteria, including *Escherichia coli*, induce disease after having attached to a particular epithelium (Conway *et al.* 1990). Indigenous bacteria may normally interfere with the colonisation of the small intestine (Freter 1992) by the *Escherichia coli* associated with PWD but following an abrupt change in diet they may not be able to compete (McAllister *et al.* 1979).

When piglets are weaned early on to dry feed they may go for long periods without eating or drinking and this could be one of the factors which predisposes them to PWD. Morishita and Ogata (1970) demonstrated that fasting in pigs caused marked changes in the gut flora. They observed that when pigs were deprived of food for 24 hours, the numbers

of *Lactobacilli* and *Bifidobacterium* in the stomach and anterior jejunum decreased markedly.

Manners (1970) found that if piglets were allowed to eat as much as they wished of a liquid diet on a twice daily feeding regimen this often led to PWD. This would suggest that in order to maintain a microflora balance in the GI tract of newly weaned pigs it would be preferable to feed frequent, small, meals. In this way the transition from sow's milk to an artificial diet would be less likely to unsettle the natural microflora.

Diet can influence the types of microbes that can colonize the GI tract of pigs. Krause, Easter, White and Mackie (1995) studied the effect of different diet regimes on the ecology of *Lactobacillus* in the GI tract of piglets. They used the Shannon, Simpson and Evenness diversity indices to demonstrate that the diversity of *Lactobacillus* is altered by the form of the diet fed to weanling pigs (Table 1.8).

Robinson *et al.* (1981) characterised the caecal bacteria of weaned pigs (age not specified) and found that 78% were gram negative and approximately 12% were gram positive. Conversely, Russell (1979) isolated bacteria from various sites of the GI tract in growing pigs and found that over 90% of the bacteria were gram positive.

In reviewing the limited data available on the development of the microflora in the GI tract of the pig it is clear that a variety of different species may be present at different times and in different circumstances. This is because studies on the total microflora are limited and conflict with each other as in the case of Russell (1979) and Robinson *et al.* (1981) even when strict anaerobic techniques are used. It may also depend on numerous other factors such as diet, environment and disease status of the herd.

Some researchers have also pointed out that populations from the GI tract of piglets vary between littermates (Kenworthy and Crabb 1963; Allison *et al.* 1979).

Table 1.8 Relative abundance of *Lactobacillus* (L) spp. obtained from the pars oesophageal of weanling pigs of different ages and consuming different diets

	Treatment[a]			
Taxon[b]	PW (5)	Sow (16)	CSL (16)	CS (22)
L. brevis	20	25	18.8	22.7
L. collinoides				
L. fermentum	20		50	27.3
L. intestinalis		12.5	6.3	
L. minutus				4.5
L. murinis		6.3		
L. oris	40	43.8	18.8	31.8
L. plantarum	20			
L. rimae		6.3		
L. uli		6.3		9.2
L. vaccinostercus			6.3	4.5

[a] Treatments bacteria cultured from 28-d-old pigs post weaning;

[b] The relative abundance of *Lactobacillus*; Figures in parenthesises indicate number of pigs.
(PW) bacteria cultured from pigs remaining on the sow from 28 to 38 days of age and receiving sow's milk as the only source of nutrition;
(Sow) bacteria cultured from pigs weaned from the sow at 28 days of age and receiving a corn-soy diet with (CSL) or without (CS) lactose until 38 days of age;
Adapted from (Krause *et al.* 1995).

Most research on the microflora of the pig GI tract has concentrated on identifying *Lactobacillus* spp. because of their known benefits to the host. It is also apparent that in those studies which do evaluate more species very small numbers of pigs were used. For example, Robinson *et al.* (1981), McGillivery and Cranwell (1992) and Russell (1979) only sampled 3, 1 and 4 pigs for each assessment respectively. To provide more insight it would be necessary to sample a much larger population of pigs.

There is little information on the changing populations of the microflora in the GI tract of the piglet, although it can be demonstrated that the microflora of the piglet is influenced

by its environment and any material which it ingests. It has also been demonstrated that starvation, infrequent feeding and diet composition have an effect on the balance of the natural microflora. Therefore, in order that the piglet microflora maintains a stable and healthy microflora which will benefit it's host, the newly weaned piglet must receive a diet which is correctly presented and consistent.

1.6 The digestive development of the piglet

The digestive development of the pig has been reviewed in detail (Walker 1959b; Aumaitre and Corring 1978; Kidder and Manners 1978) and Low (1980), enzyme development by (Bailey, Kitts and Wood 1956; Kitts, Bailey and Wood 1956; Morgan and Robinson 1962; Aumaitre 1971; Kidder and Manners 1980) and Cranwell (1995) and the affects of weaning on the digestive system by (Hartman, Hays, Baker, Neagle and Catron 1961; McCracken and Kelly 1993; Partridge and Gill 1993; Aumaitre, *et al.* 1995; Nabuurs 1995).

The piglet is born with an immature digestive system, has limited body reserves and is highly dependent on the sow to provide adequate nutrients (Harrell, Thomas and Boyd 1993). It is fully adapted to utilise sow's milk (Sangild, Cranwell, Sorensen, Mortensen, Noren, Wetteberg and Sjostrom 1991), the composition of which changes gradually during lactation (Table 1.9). Initially the piglet ingests colostrum, which contains a high concentration of total solids and protein but only low levels of fat and lactose (Pluske, *et al.* 1995). During the first two or three days of lactation a transition occurs from colostrum to milk which consists of approximately 65% fat 22% protein, and 14% lactose (Table 1.10). The constituents of sow's milk are highly digestible by the young pig which is adapted to digest sows milk with almost 100 per cent efficiency (Pluske *et al.* 1995). In order to do this the suckling piglet secretes large amounts of pancreatic lipase, lactase and several proteolytic enzymes (Fowler 1985).

Table 1.9 Changes in gross composition of sow's colostrum and milk over time

	Time after parturition (hours)					(weeks)
	0	6	12	27-48	72-120	2-8
(g kg^{-1})						
Total solids	302	266	208	212	218	212
Fat	72	78	72	95	104	93
Protein	189	152	102	69	68	62
Lactose	25	29	34	40	46	48
Ash	6.3	6.2	6.3	7.2	7.7	9.5
Calcium	0.5	0.5	0.6	1.1	1.6	2.5
Phosphorus	1.1	1.1	1.1	1.3	1.4	1.5

After (Perrin 1955).

Table 1.10 The main components of colostrum and sows milk and their relative contribution to gross energy

	Fresh sample g kg^{-1}	% of total gross energy
Colostrum (3 hours after farrowing)		
Total crude protein	175	56.5
Total lipids	67	36.1
Lactose	32	7.4
Milk (7 day after lactation)		
Total crude protein	56	21.5
Total lipids	101	65.0
Lactose	49	13.5

Adapted from (Pluske *et al.* 1995).

Digestion and absorption of nutrients takes place throughout the alimentary tract of the piglet by mechanical, chemical and microbial processes (McDonald, Edwards and Greenhalgh 1988). The components of the digestive system which are mainly responsible for the digestion and absorption of nutrients are the stomach, small intestine, pancreas and liver (Cranwell 1995). The ability of the piglet to carry out digestive and absorptive functions will depend on gut capacity, the nature and quantity of the secretions it can provide, the development of mechanisms to control these secretions, and the digestive and absorptive capacity of the mucosal surface of the small intestine (Cranwell 1995).

During the time that the piglet is suckling gradual changes take place in the digestive system. This is part of a natural process which enables the young piglet to adapt from the declining quantity of sow's milk to other feed sources which are much less digestible than sow's milk (Kidder and Manners 1978). This gradual process reduces the likelihood of digestive upsets arising from the inability to utilise new feed sources (Kidder and Manners 1978). Unfortunately for the piglet, in modern production systems weaning is not a gradual process but rather a very abrupt process where the diet is switched from a liquid based mainly on fat, protein and lactose to a dry diet based mainly on starch and less digestible animal proteins (Close 1993). In this respect, weaned piglets require more developed digestive tracts than the suckling pigs.

The stomach is the first major site of digestion where food particles are degraded in the presence of gastric secretions of hydrochloric acid (HCl). Although sow's milk has a high buffering capacity it does not strongly stimulate the secretion of HCl (Kidder and Manners 1978). According to Bolduan *et al.* (1988) HCl production occurs from birth but is inhibited by the presence of lactic acid during the suckling period. This view is in agreement with that of Cranwell (1995) who concluded that the presence of lactic acid,

(produced in large amounts in the stomach of suckled pigs) may partly, or completely inhibit acidification by HCl. During suckling a low pH is maintained by the presence of lactic acid and this source is removed at weaning causing a rise in stomach pH (Bolduan *et al.* 1988). Dry food can cause a rapid rise in pH because of the piglets limited capacity to secrete HCl. A stomach pH above 4.0 results in reduced proteolysis because pepsinogen (a pre-cursor of pepsin) is inhibited, (Kidder and Manners 1978). This often results in undigested material of a high pH reaching the intestines where it provides both a pH and nutrient substrate suitable for the multiplication of enteropathogenic bacteria (Smith and Jones 1963; Kidder 1982; Bolduan *et al.* 1988; Hill and Sainsbury 1995) the optimum pH of which are presented in table 1.11.

Table 1.11 Approximate pH ranges of microbial growth

Organism	Minimum	Optimum	Maximum
Clostridium perfringens	5.0 5.5	6.0 7.6	8.5
Escherichia coli	4.3 4.4	6.0 8.0	9.0 10
Salmonella (most)	4.5 5.0	6.0 7.5	8.0 9.6

After (Banwart 1989).

Therefore, in designing diets for young pigs two approaches may be taken to overcome this problem; either dietary compounds with low acid binding capacity must be selected or the diet must be acidified. Bolduan *et al.* (1988) pointed out the importance of taking into consideration the acid binding capacity of weaner diets in order to account for the limited ability of piglets to secrete sufficient HCl. An indication of the acid binding capacity of some common feed ingredients is presented in table 1.12.

Table 1.12 Consumption, of hydrochloric acid required to reach pH 4 (nmol 100 g^{-1} of original material)

Skim milk	3.07
Skim milk, fresh	7.12
Wheat	8.99
Barley	9.97
Yeast	30.10
Soyabean, extracted and toasted	50.68
Fish meal	60.38
Skim milk, dried	66.37
Mineral mixture	1260.50

After (Bolduan *et al.* 1988).

A number of researchers have investigated the effect of acidification of piglet diets to support digestion in the stomach (Table 1.13). From the data presented in table 1.13, it is clear that the acidification of diets for young piglets generally improves pig performance when compared to those piglets fed the same diets without the addition of organic acids. Henry, Pickard and Hughes (1985) observed that citric acid stimulated food intake in young piglets and as a result growth rates were improved. He suggested that these improved growth rates may have resulted from a reduction in bacterial competition for nutrients. Earlier experiments by Kershaw, Luscombe and Cole (1966) revealed that adding lactic acid to the drinking water of starter pigs resulted in a lower number of coliform bacteria in the GI tract in the immediate period post weaning.

It is possible that the use of organic acids helps to maintain the lactic microflora of the piglet post weaning and in this way protects the piglet from enterpathogenic infection. For example, Mathew, Sutton, Scheidt, Forsyth, Patterson and Kelly (1991) reported that propionic acid tended to reduce coliform bacteria and increase *Lactobacillus* concentrations in the upper portions of the GI tract of young piglets.

Table 1.13 The effect of the addition of organic acids to dry piglet diets on overall weight gain[a]

Reference	Age of pig		Control	Lactic	Fumaric	Citric	Formic
(Henry *et al.* 1985)	10	(d)	100	-	89	114	-
(Risley, Kornegay, Lindemann and Weakland 1991)	1 - 4	(wk)	100	-	103	108	-
(Bolduan *et al.* 1988)	3	(wk)	100	112	113	-	113
(Risley *et al.* 1993)	3 - 4	(wk)	100	-	112	114	-
(Roth, Kirchgessner and Eidelsburger 1993)	3 - 6	(wk)	100	108	-	-	-
(Radecki, Juhl and Miller 1988)	4 - 6	(wk)	100		126	100	-
(Edmonds, Izquierdo *et al.* 1985)	4 - 7	(wk)	100	-	106	130	-
(Krause, Harrison and Easter 1994)	4 - 8	(wk)	100	-	110	105	-
(Falkowski and Aherne 1984)	4 - 8	(wk)	100	-	104	107	-
(Geisting and Easter 1985)	4 - 8	(wk)	100	-	104	103	-
(Kershaw *et al.* 1966)[b]	8 - 10	(wk)	100	126	-		-

(d) days; (wk) weeks.
[a] expressed as a percentage of the control
[b] lactic acid was added to the drinking water only

Other workers such as Risley, Kornegay, Lindemann, Wood and Eigel (1993) and Eidelsburger (1996) have suggested that organic acids, such as fumaric acid, may also serve as an energy source for the weanling pig, since fumaric acid has been shown to have an energy value equal to that of glucose. Several researchers have suggested that there may be improved protein utilization when starter diets are supplemented with fumaric acid (Geisting and Easter 1985; Radecki *et al.* 1988) and Kirchgessner and Roth (1982). The use of organic acids may indeed affect the way in which proteolysis occurs because the four pepsins produced in the stomach of the pig have optimum activities at two different pH levels (2.0 and 3.5) (McDonald *et al.* 1988; Eidelsburger 1996). Thus lowering the pH of the stomach to the optimum range for the activity of pepsinogen may increase the rate of cleavage of peptide bonds which normally occurs in the stomach as a result of the presence of HCl. However, newly weaned pigs fed on dry diets rarely secrete enough HCl to maximise proteolysis (Kidder and Manners 1978) and as a result undigested food may enter the duodenum at a higher pH. It is the presence of acid and food in the duodenum which stimulates the hormones secretin and cholecystokinin to be liberated from the mucosa. Both these hormones stimulate the pancreas to release pancreatic juice which contains a mixture of enzymes and other chemicals (McDonald *et al.* 1988). Undigested food of a higher pH may fail to trigger the signals for the secretion of pancreatic juice and consequently allow the substrate to pass into the small intestine almost intact.

(Hill and Sainsbury 1995) have pointed out that acids are added to creep feeds but not usually to post weaner diets. Discussions with feed company personnel suggest that this may not be a true reflection of current manufacturing processes and that the use of acids in post weaning diets is widespread. If there is any justification for acidifying creep feeds then it is even more important to consider acidifying post weaned piglet diets because of the sudden withdrawal of lactic acid at weaning time. There is also the possibility that at

weaning the normal microflora may be suppressed by the multiplication of pathogenic bacteria which also excacerbates the situation.

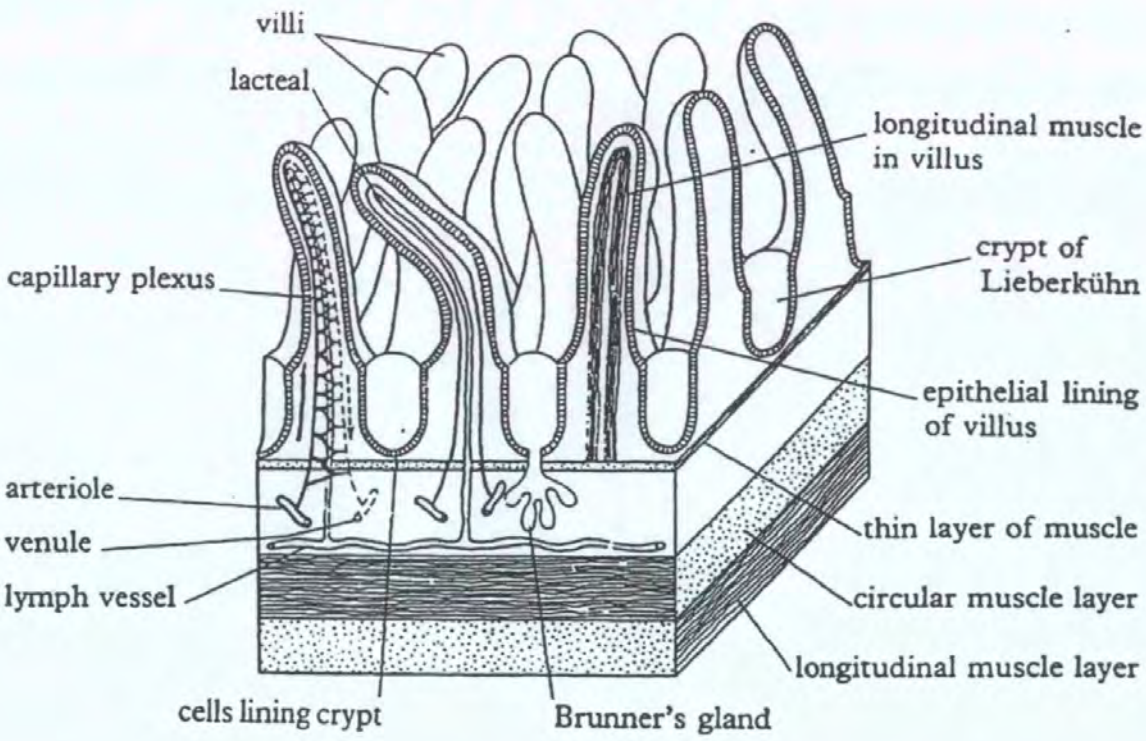
1.7 Effect of weaning on the morphology of the digestive system of the piglet

The partially digested food leaving the stomach enters the small intestine where it is mixed with secretions from the duodenum, liver and pancreas. Bile salts made by the liver and secreted by the gall bladder, are involved in the activation of pancreatic lipase and emulsification of fats. The surface of the small intestine is covered with villi which increase the surface area for the absorption and digestion of nutrients (Figure 1.3). The crypt cells migrate to the villus tip secreting enzymes in the process (Roberts 1986). The small intestinal villus epithelium is replaced after 7 - 10 days in day old pigs and 2 - 4 days in 3 week old pigs (Moon 1971). Secretory cells situated in the crypts of Lieberkuhn in the wall of the small intestine produce mucous and a variety of enzymes.

The morphology of the small intestine changes after weaning; in particular there is a shortening of the villi and deepening of the crypts of Lieberkuhn. Hampson (1986b) and Miller, James, Smith and Bourne (1986) demonstrated that weaning had a dramatic effect on the structure of the villus and crypt depth.

Miller *et al.* (1986) conducted a comprehensive study on the effect of weaning on the capacity of pig intestinal villi to digest and absorb nutrients. Their results clearly demonstrated that weaning affected gut morphology. Villus length, which did not change significantly in unweaned piglets 4 - 6 weeks after birth, was halved 5 days after weaning, and crypt depth, which increased normally in unweaned piglets, was further increased by weaning (Table 1.14).

Figure 1.3 Cross section of the small intestine showing villus architecture.



After Roberts (1986)

Table 1.14 The effect of age and weaning upon piglet intestinal structure

Age (weeks)	Location[a] (% length)	Villus height (μm) unweaned	Villus height (μm) weaned	Crypt depth (μm) unweaned	Crypt depth (μm) weaned
4	25	628	330	169	320
	50	681	359	161	344
6	25	561	310	268	326
	50	639	349	269	358

[a] measurements made on pieces of small intestine taken 25 and 50% along the intestinal tract of 4 and 6-week-old clean piglets before 5 days after weaning.
Adapted from (Miller *et al.* 1986).

In the study by Hampson (1986b) unweaned pigs showed a gradual increase in crypt depth which occurred with age whereas weaned pigs exhibited a highly significant increase in crypt depth and reduction in villus height (Table 1.15). It has been suggested that these changes lead to reduced digestive and absorptive capacities and may be one factor which causes a post weaning growth check in newly weaned pigs fed on dry diets. It has been suggested by Smith (1984) and Hampson (1986b) that crypt depth is an indicator of the rate of crypt cell proliferation. An increase in crypt depth implies an increased rate of enterocyte production and migration which means that the enterocytes have insufficient time to express maximal brush border enzyme activity before being extruded from the tip of the villus.

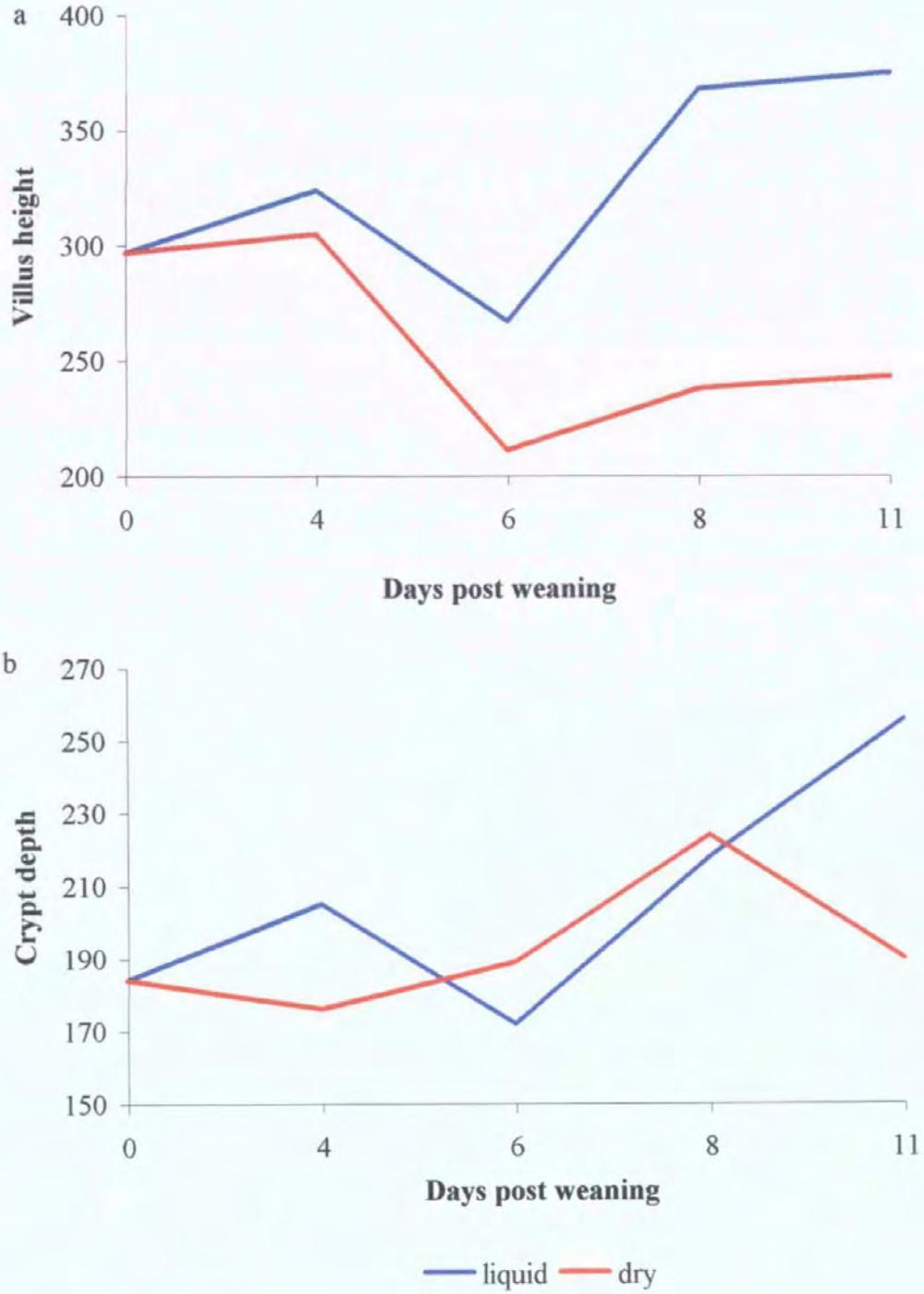
The presentation of the diet post weaning has also been shown to affect gut morphology. For example, Deprez, Deroose, Vandenhende, Muyelle and Oyaert (1987) conducted an experiment which compared the effect of liquid feeding or dry feeding on the gut morphology of newly weaned piglets. They demonstrated that liquid feeding may be important in preserving villus height and crypt depth (Figure 1.4 a,b).

Table 1.15 Changes in the morphology of the small intestine of piglets over time.

Group	weaned or not	creep feed or not	Age in days 21	22	23	24	25	26	29	32	Sig
Villous height[a]											
3	+	-	-	643	494	554	433	369	515	507	P<0.001
4	-	-	885	783	652	686	623	551	523	458	P<0.01
Crypt depth[a]											
3	+	-	-	134	176	128	205	229	304	281	P<0.001
4	-	-	119	96	107	131	117	173	150	128	P<0.05

[a] mean values measured in micro metres at a site 75 % along the small intestine measured
Adapted from Hampson 1986b.

Figure 1.4 The effect of liquid or dry feed on the morphology in the ileum of post weaned piglets.



Adapted from Deprez *et al.* 1987

Some researchers have postulated that changes in gut morphology at weaning could be a result of hypersensitivity to dietary antigens such as those found in soyabean which may cause an inflammatory response (Hall and Byrne 1980; Miller, *et al.* 1984a; Newby, *et al.* 1985). However, more recent studies (Hampson, *et al.* 1988; Kelly, O'Brien and McCracken 1990) have examined this hypothesis but failed to reproduce the hypersensitivity response. Li *et al.* (1991) demonstrated that the type of protein in the diet affects gut morphology and xylose absorption. However, on re-examination of the performance data it can be seen that pigs fed milk protein had higher feed intakes than those fed soya-protein and consequently the higher villus heights in milk fed piglets may have been a response to higher feed intakes rather than reduced dietary antigens, previously presented in table 1.5.

1.8 The effect of nutrient intake on the morphology of the digestive system of the piglet.

Recent evidence suggests that the nutrient intake in the newly weaned piglet may be of great importance in promoting the development of the digestive system and in particular maintaining the integrity of the gut. McManus, Kurt and Isselbacher (1970) have stated that

> 'the small intestine exhibits an unrivalled rate of cell turnover and was remarkably sensitive to alterations in its micro-environment. Any interference with either the pattern of feeding, the quality or quantity of food, or the rate of food ingestion may result in significant disturbances in intestinal structure and function'.

If nutrient intake is considered to be of great importance then it might be expected that starvation would have the opposite effect, and indeed earlier research on nutrient intake would support such an hypothesis. For example McManus *et al.* (1970) and Steiner, Bourges, Feedman and Gray (1968) examined the effects of starvation on the small

intestine in rats and demonstrated that there was a reduction in mucosal mass and a depression in enzyme capacity as a result of malnourishment. Steiner *et al.* (1968) demonstrated that during periods of starvation weight loss of the small intestine is out of proportion to body weight loss. Subsequently Rudo, Rosenberg and Wissler (1976) examined the effect of partial starvation on the intestinal villus and cell migration in rats. He demonstrated that mean villus length in semi-starved rats was significantly reduced ($P<0.001$), and the rate of cell migration diminished compared to control animals (Table 1.16).

Table 1.16 The effect of semi starvation treatment on villus length and crypt depth in the small intestine of rats

Treatment	Villus length (mm)	Crypt depth (mm)	n	Significance
Control	0.75	0.19	15	-
Semistarved	0.66	0.16	14	$P<0.001$

Adapted from (Rudo *et al.* 1976).

Whilst some researchers have demonstrated the effects of nutrient deprivation on the gut morphology of the piglet, other researchers have investigated the effects of maintaining nutrient intake. For example, Kelly, Smyth and McCracken (1991b) have demonstrated that nutrient intake in the weaned pig affects the anatomy, morphology and function of the gut. They investigated the effect of the level of food intake on the digestive development of the newly weaned pig and demonstrated that villus height was significantly higher ($P<0.001$) and crypt depth was significantly higher ($P<0.05$) in piglets fed a continuous nutrient supply (Table 1.17).

Table 1.17 Villus height and crypt depth at sites 1, 3 and 5 of the small intestine of weaned pigs given continuous or restricted nutrient supply

	Villus height (μm)		Crypt depth (μm)	
	Continuous	Restricted	Continuous	Restricted
Site 1	546	404	197	182
Site 3	481	437	211	168
Site 5	390	313	214	191

Adapted from (Kelly *et al.* 1991b).

Recent research by Pluske *et al.* (1996a) has investigated whether maintenance of nutrition after weaning would prevent the normal decline in villus height and increase in crypt depth. They demonstrated that piglets weaned on to a dry diet fed *ad libitum* had shorter villi ($P<0.001$), deeper crypts ($P<0.001$), and proportionally (0.21 to 0.28) less protein ($P<0.05$) in their intestinal mucosa, than piglets weaned on fresh ewes' milk. Their results revealed a significant relationship between total dry matter intake and villi height at the proximal jejunum in piglets given the starter diet ($r = 0.78$, $P = 0.039$) and ewes' milk ($r = 0.65$, $P = 0.073$) (Table 1.18). Pluske *et al.* (1996a) concluded that maintaining nutrition after weaning at levels approximating pre-weaning intake prevents villus atrophy. It is interesting to note that in their experiment the piglets fed on fresh ewes' milk began eating within 8 hours of weaning thereby avoiding a prolonged period of starvation. In a further study (Pluske *et al.* 1996b) piglets weaned at 28 days were fed *ad libitum* either a dry starter diet, fresh cows' milk at maintenance energy intake, fresh cows' milk at 2.5 maintenance or fresh cows' milk fed *ad libitum*. A positive correlation was found between voluntary food intake and both villus height and crypt depth indicating that one of the factors in maintaining mucosal integrity is nutrient intake (Figure 1.5).

Table 1.18 Villus height and crypt depth of piglets killed at weaning or 5 days later

	Proportion of intestine	Treatment SR	Starter	EM	Significance
Villus height (μm)	0.25	550[a]	330[b]	569[a]	***
	0.50	464[a]	316[b]	428[a]	**
	0.75	264	261	258	
Crypt depth (μm)	0.25	116[a]	182[c]	137[b]	***
	0.50	124[a]	179[b]	137[a]	***
	0.75	97[a]	172[c]	123[b]	***
Performance of piglets after weaning					
Live weight (kg)					
weaning		8.6	8.9	9.1	
after 5 days			10.4	11.3	
Daily gain (g d^{-1})					
live weight			307[c]	435[d]	**
Voluntary food intake (g dry matter d^{-1})			320	295	
Energy intake (MJ GE d^{-1})			5.7[c]	7.4[d]	***

[a,b,c,d] Within rows, means not followed by a common superscript differ significantly.
* P<0.05; ** P<0.01; *** P<0.001.

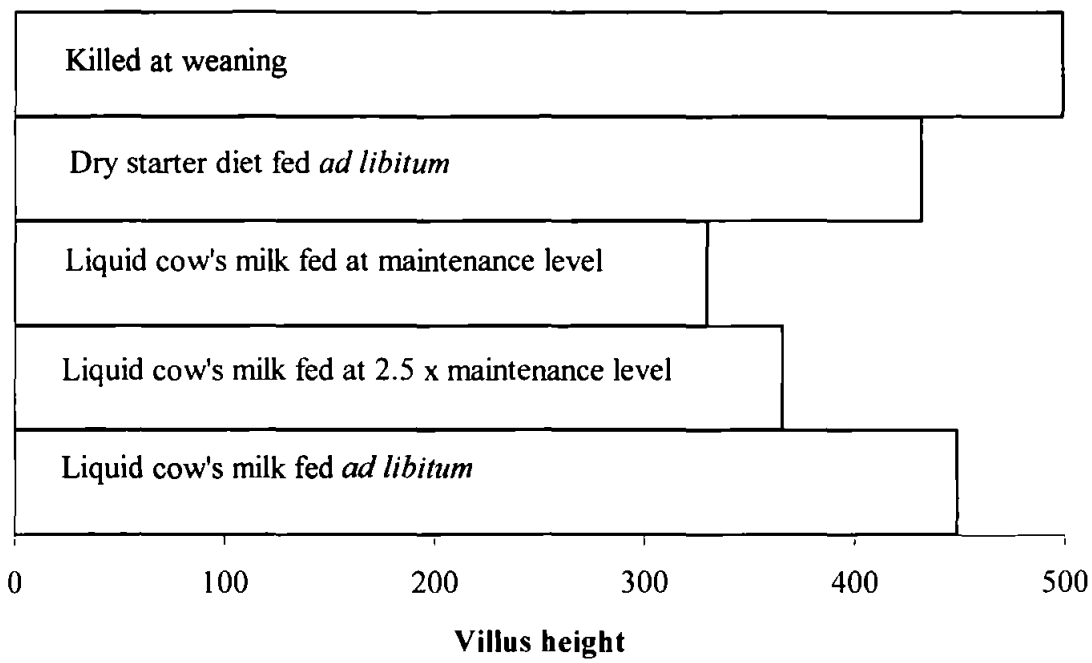
SR piglets killed at weaning;

Starter piglets given dry starter diet *ad libitum*;

EM piglets given fresh ewes' milk every 2 hours for 5 days.

After (Pluske *et al.* 1996a).

Figure 1.5 Relationship between nutrient intake and villus height in the small intestine of weaned piglets.



Adapted from Pluske *et al.* 1996b.

It can be concluded from their studies that the absence of nutrients from the lumen caused a change in the gut morphology which in turn affected growth performance. Weaning is often associated with periods of starvation which would deprive the gut mucosa of nutrients. Therefore, it is important that newly weaned piglets receive an adequate supply of nutrients in order to maintain their mucosal integrity.

Liquid feeding has been shown to be a successful method of improving voluntary feed intake (VFI) in weaned piglets. Partridge *et al.* (1992) have demonstrated that piglets weaned at 23 days of age and fed liquid diets had a significantly greater VFI (41 g d^{-1} $P<0.05$), than piglets fed dry diets (Table 1.19).

Table 1.19 Intakes and growth rates of weaned piglets on a conventional dry or a liquid feeding system

	Dry feed	Liquid feed	Significance
Intake (g d^{-1})			
Week 1	149	176	< 0.1
Week 2	327	357	NS
Week 3	453	518	< 0.1
Overall	310	351	< 0.05
Growth rate (g d^{-1})			
Week 1	133	147	NS
Week 2	330	355	< 0.05
Week 3	380	434	< 0.001
Overall	281	312	< 0.01

After (Partridge *et al.* 1992).

Liquid feeding may also enhance gut health and function by providing appropriate conditions for enzyme activity, digestion, nutrient absorption and microbial growth. Morgan and Robinson (1962) suggested that the effect of low enzyme activity on the digestibility of a nutrient was influenced by saturation of the enzyme by its substrate. In a liquid diet, enzyme activity should be enhanced due to the separation of the solid feed

which provides smaller particles for direct absorption, and presents a greater surface area for enzyme action. For example, if lipids are emulsified to particle size of less than 0.5 microns they can be absorbed directly by the lacteal (Morgan and Robinson 1962).

1.9 The effect of age and weaning on the development of enzyme systems in newborn piglets

During the first few weeks of the piglets life there are changes in the enzyme systems which affect the ability of the piglet to digest nutrients in the feed. Due to the variations in the diets fed to piglets as creep feed and post weaning there can be no simple description of the general developmental pattern of enzymes. However, a number of researchers have investigated the development of enzyme systems in piglets and the results of their research gives an indication of the general pattern of development of the enzyme systems of the piglet (Table 1.20).

Pigs are not born with a full compliment of enzymes. The enzyme systems in the young pig are appropriate for the digestion and assimilation of sow's milk. In contrast the adult animal possesses an enzyme system which enables it to digest a wide range of feed materials. In nature the development of the enzyme system occurs in parallel with the pigs transition from sow's milk to solid food. In contrast the domesticated pig, weaned at 3 - 4 weeks of age must make a rapid transition from sows' milk to solid feed before it possesses a full compliment of enzymes. To help or overcome this problem nutritionists can adopt two approaches. First, the selection of highly digestible raw materials which the piglet is capable of assimilating with its under developed enzyme system and secondly the supplementation of diets with enzymes which compliment and make up for deficiencies in the piglets own enzyme compliment.

Table 1.20 Summary of the changes in the development of the enzyme systems of suckling piglets from birth to maturity

Reference	Enzyme	Observed changes
(Walker 1959b) and (Kitts *et al.* 1956)	Pancreatic amylase	Negligible at birth increasing rapidly up to 5 weeks of age
(Morgan and Robinson 1962)		Marked increase during the first 10 days of life
(Walker 1959b)	Intestinal amylase	Remained constant from 1 week to 5 weeks of age
(Morgan and Robinson 1962)		Marked increase during the first 10 days of age
(Aumaitre 1971) and (Kitts *et al.* 1956)	Pancreatic lipase	High activity from birth, remaining steady during the suckling period
(Hartman *et al.* 1961)	Stomach proteinase	Birth to 2 weeks decreasing, then increasing from 2-7 weeks of age
(Aumaitre 1971)	Pancreatic protease	Varied according to age
(Walker 1959b)	Pancreatic sucrase	Negligible at birth, increasing to high levels at 5 weeks of age
(Manner and Stevens 1972)	Intestinal sucrase	Increasing from birth to 7 weeks
(Bailey *et al.* 1956)		From 1 week of age levels continued to rise until maturity
		Negligible at birth reaching a maximum after 25 days
(Walker 1959b)	Pancreatic maltase	Negligible at birth, increasing to high levels at 5 weeks of age
(Bailey *et al.* 1956)	Intestinal maltase	Negligible at birth reaching a maximum after 25 days
(Hartman *et al.* 1961)		Gradual increase from the first week after birth to 7 weeks of age
(Morgan and Robinson 1962)	Intestinal lactase	High at birth falling rapidly over time
(Hartman *et al.* 1961)		From birth to 3 weeks a rapid decrease then remaining constant
(Manner and Stevens 1972)		High in birth declining over 8 weeks
(Bailey *et al.* 1956)		High at birth declining to a minimum at 3 - 4 weeks of age

Weaning has a marked effect on the pattern of enzyme development in the young piglet because early weaned piglets lack their full compliment of enzymes and their digestive system has not reached maturity. Maturity of the digestive system means "the ability, which is possessed by the adult pig, to digest a wide range of different foodstuffs" (Kidder 1982). The drastic change in diet which occurs at weaning demands that the piglet possess appropriate stomach secretions, pancreatic and intestinal enzymes which are not fully developed during the suckling period (Aumaitre, *et al.* 1995).

Feed manufacturers currently add exogenous enzymes to weaner diets to help overcome deficiencies. The use of exogenous enzymes to pig diets has previously been reviewed (Rotter 1990; Classen and Bedford 1991; Chesson 1993; Dierick and Decuypere 1994; Officer 1995). Officer (1995) presented data from a review of published work which examined the responses of weaner piglets to dietary enzyme supplementation some of which are presented in table 1.21. Kidder and Manners (1980) suggested that the pattern of carbohydrase in the small intestine of piglets did not approach that of an adult pig until at least 8 weeks of age. In their study sucrase, isomaltase, trehalase and glucoamylases increased with age whilst lactase activity decreased. Hampson *et al.* (1986a) demonstrated that weaning pigs at 3 weeks of age resulted in a large and rapid reduction in lactase activity in the small intestine and that sucrase activity declined temporarily but then recovered (Table 1.22).

Hartman *et al.* (1961) described the development of digestive enzymes in suckled and weaned pigs (< 21 days of age). They found that pancreatic protease activity was greater in sow-reared pigs than in pigs weaned to a dry diet; however, the enzyme activity in the intestinal contents was higher in weaned pigs.

Table 1.21 Review of published responses of weaner piglets to dietary enzyme supplementation

Reference	Enzyme	Diet	Response
Suga *et al* 1978[a]	Cellulase	Wheat/maize/fish/soya/rice bran	+ 45% DG, + 9% FCR
Thomke *et al* 1980[a]	B-glucanase	Enzyme pre-treated barley/ protein concentrate	+ 5% DG, + 5% FCR
Bohme 1990[a]	Cellulase/B-glucanase/ amylase/glucoamylase	1. barley/wheat 2. barley/wheat/maize	+ 10% FCR, less diarrhoea + 15% FCR, less diarrhoea
Inborr and Graham 1991	B-glucanase	barley/soya/SMP	+ 17% DG, nil effect on FI
Hogberg *et al* 1983[a]	Amylase/sucrase	maize/soya	Nill effect on DG, FCR
Inborr and Ogle 1988[a]	Amylase/B-glucanase/ glucoamylase	cooked barley enzyme pre-treated/oats/soy/fish	Nil effect on DG and FCR, less diarrhoea
Bedford 1992	Xylanase	Rye/soya	Nil effect on DG, FCR
Thacker *et al* 1992[a]	B-glucanase	barley/soya	Nil effect on DG, FI

[a] cited by Officer, 1995.
DG = Daily gain; FCR = Feed conversion ratio; FI = Feed intake

Table 1.22 Changes in the enzyme activities in the small intestine of piglets over time.

Group	weaned or not	creep feed or not	Age in days 21	22	23	24	25	26	29	32	Sig
Lactase[a]											
1	+	+	-	129	122	94	56	48	47	79	P<0.001
2	-	+	227	250	196	169	183	139	107	131	P<0.05
Sucrase[a]											
1	+	+	-	66	49	32	23	36	20	64	P<0.001
2	-	+	67	83	93	77	70	80	71	75	ns

[a] Specific enzyme activity iu g^{-1} mucosal protein
Adapted from Hampson and Kidder 1986a.

(Owsley, Orr and Tribble 1986) investigated the effect of age and diet on the development of the pancreas and secretion of pancreatic enzymes in young pigs from birth to 56 days of age. Piglets were offered creep feed at 14 days and were weaned at 28 days. Enzyme activities in the intestinal contents and pancreas were low following weaning. However, increasing feed intake immediately after weaning increased the amount of pancreatic enzymes synthesized and secreted, thereby improving feed performance. They concluded that while the pig has the enzyme capabilities to digest a typical starter diet some problem inhibits the release of enzymes from the pancreas. The results indicate that increasing feed intake immediately after weaning will increase the amount of pancreatic enzymes synthesized and secreted, thus improving pig performance (Table 1.23).

Table 1.23 The effects of age on the development of pancreatic enzyme activity in piglets weaned at 28 days of age

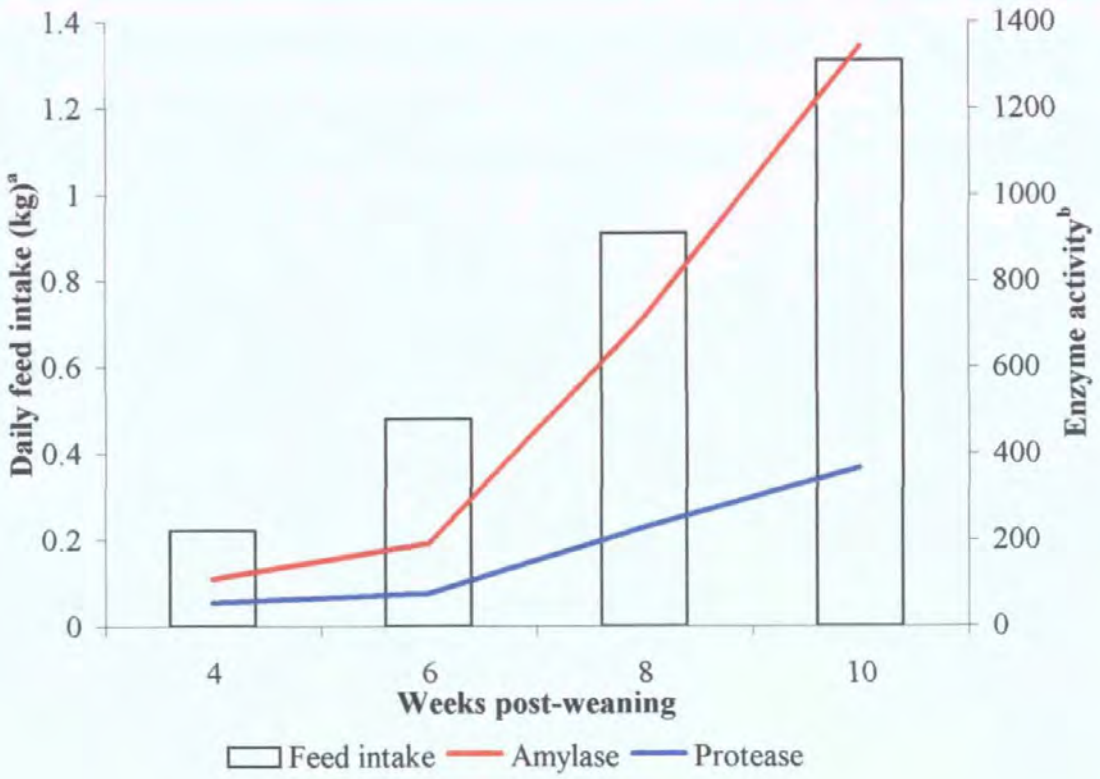
Age (days)	Trypsin[a]	Chymotrypsin[a]	Amylase[a]
0	5526	259	429
14	2325	230	11074
27	4712	324	21864
29	5885	325	29299
31	5553	100	8958
42	24497	257	27250
56	38668	320	40959

[a] measured in units per gram of pancreas homogenate
Adapted from (Owsley *et al.* 1986).

Shields, Ekstrom and Mahan (1980) agreed with Owsley *et al.* (1986) that increasing feed intake post weaning increased the total activity of protease and amylase in piglets. However, they concluded that neither weaning age nor feeding method pre- or post weaning influenced long term enzyme development or pig performance. More recent research by Makkink, Negulescu, Guixin and Verstegen (1994) has demonstrated that feed intake in piglets was very important for the development of digestive enzyme activity. They concluded that it was not the piglets lack of potential enzyme secretion but higher feed

intakes which stimulated the release of higher rates of pancreatic enzymes secretion. In view of the work of Makkink *et al.* (1994), Shields *et al.* (1980), data can be reinterpreted and it can be seen from the data in figure 1.6, that nutrient intake may be an important factor in stimulating enzyme activity.

Figure 1.6 The effect of feed intake (paste form) on enzyme activity of piglets weaned at 2 weeks of age.



[a] Air dried basis

[b] Enzyme acitivity is expressed as starch hyrolyzed/minute for amylase and mg tyrosine equivalents produced/minute for protease.

Adapted from the data of Sheilds *et al.* 1980.

1.10 The effect of nutrition on the enzyme systems of the piglet.

There is considerable evidence that the development of enzymes is stimulated as a result of the substrate the piglet consumes (Kitts, *et al.* 1956; Kim, Benevenga and Grummer 1978; Graham, Mahan and Shields 1981; Efird, Armstrong and Herman 1982a; Efird, *et al.* 1982b; Owsley *et al.* 1986; Makkink *et al.* 1994). For example Makkink *et al.* (1994) investigated the effect of dietary protein source on pancreatic enzyme activities in newly weaned pigs and reported that protein source affected pancreatic and jejunal trypsin and chymotrypsin activities. At day 3 of the feeding trial, the highest trypsin activity was found in piglets fed on a skimmed-milk powder diet and the lowest activity was found in piglets fed on soya-bean meal and fish-meal. At day 3, total chymotrypsin activity was higher for fish meal fed piglets than for the soya-bean meal fed piglets. Manner and Stevens (1972) observed that baby pigs, reared artificially on diets containing sucrose, had higher specific sucrase activity in the small intestinal mucosa. Subsequently, McCracken (1984) noticed that a greater increase in maltase activity occurred in pigs weaned at 14 days which had consumed large quantities of cereal-based diets compared with sow reared pigs. They suggested that the nature of the dietary carbohydrate can alter the extent of enzyme induction in the immediate post weaning period. Efird *et al.* (1982b) demonstrated that piglets weaned at 21 days and fed a soy protein diet tended to have greater trypsin and chymotrypsin activities in the intestinal content, and a larger weight of pancreas than those pigs fed milk protein diets (Table 1.24).

Table 1.24 Effects of diet on enzyme activities, and pancrease weight, in piglets aged 35 days, weaned at 21 days of age

	Milk diet (dry)	Corn-soya diet (dry)	Sig
Pancreas wt, (g kg^{-1} body wt)	1.5	2.0	(P<0.05)
Total trypsin activity[a]	10.5	36.3	(P<0.05)
Total chymotrypsin activity[b]	24.0	21.3	(P<0.05)

[a,b] Total activity represents a combination of pancreatic and small intestinal content activities. All activities are expressed in units kg^{-1} body weight where one unit of activity is defined as the amount of product released by 1 mg of a known enzyme standard.
Adapted from (Efird *et al.* 1982b).

Conclusion

The review of literature has demonstrated that the digestive system of the piglet undergoes dramatic changes from birth to weaning and again from weaning to maturity. The morphology of the small intestine is directly affected by the process of weaning and the change in its diet that occurs at that time. The enzyme systems of the piglet, which are already undergoing rapid changes from birth to weaning, are also dramatically influenced by weaning and may also be conditioned by dietary changes.

It has also been demonstrated that maintaining nutrient intake is extremely important in preserving the integrity of the gut. This highlights the practical importance of managing the young piglet in a way which maintains intake and thereby minimises the detrimental affects of weaning and dietary changes. This in turn will assist in preserving the integrity of the digestive system.

In designing diets for newly weaned pigs nutritionists have taken two approaches to the problem of lack of maturity of the digestive system of young piglets by 1) formulating diets from highly digestible ingredients, or 2) supplementing the diets with exogenous enzymes.

The review has also established that a third approach may be considered as a viable option in the management of nutrition at weaning, namely the use of a liquid feeding system. A liquid medium provides more appropriate conditions for the action of the piglets own enzymes. Liquid feeding also assists in a smoother transition from sow's milk at weaning and the literature would suggest that it should also result in less disruption of the small intestine by maintaining the nutrient supply to the gastrointestinal tract.

1.11 The affect of genetic potential, weaning weight and age on the growth performance of piglets

If the piglet is to continue to grow at it's pre-weaning rate, and maximise it's genetic potential for growth then the factors which limit this potential growth rate need to be overcome. One of the factors which influences post weaning growth rate is the age at which the piglet is weaned. Age at weaning is a major factor in the control of reproduction in sows and an important determinant of annual productivity (Aumaitre *et al.* 1995). In the wild, piglets would remain with the sow for an average of 17.2 weeks (Jensen and Recen 1989) before they were fully weaned, whereas current UK practice is to wean at between 25 days of age and 5 weeks of age (Table 1.25). Recently a minimum weaning age of 3 weeks has been recommended by European legislation (OJEC 1991), reflecting the pig producing industry's response to welfare recommendations (MLC 1994). This weaning age is presently a common practice in many countries (Aumaitre *et al.* 1995).

Table 1.25 Trends in weaning age from 1985 to 1995

Age at weaning (days)	1985	1987	1989	1991	1993	1995
< 19	5	3	2	< 1	< 1	<1
19-25	62	66	59	59	54	52
26-32	22	22	30	33	38	44
33-39	9	8	7	6	7	3
> 39	2	1	2	1	0	1

After (MLC 1994; MLC 1996).

When piglets are weaned earlier (19 - 25 days of age) as opposed to 33 - 39 days of age the numbers of litters per sow per year increases, mortality of piglets decreases and the cost of sow feed increases. However, the cost of feed per pig reared increases if pigs are weaned later (Table 1.26).

Table 1.26 The effect of age at weaning on sow productivity and feed costs

	Age at weaning (days)		
	19-25	26-32	33-39
Feed cost per pig reared (£)	9.44	9.52	11.95
Litters per sow per year	2.26	2.22	2.05
Mortality of pigs born alive (%)	11.30	12.40	13.00
Sow feed per sow per year (£)	188.62	182.37	186.58
Pigs per sow/year	11.77	11.89	10.90

After (MLC 1996).

The growth performance of the weaned piglet is determined by age and weight at weaning, the genetic potential for growth, quality of management and environment, social conditions, diet and the disease status of the animals (Aherne, Hogberg, Kornegay and Shurson 1992). The genetic potential for growth of the young piglet will have been determined by its parents, who in turn have been selected from genetic selection programmes designed to improve food conversion ratio (FCR) and lean content (Webb 1989). This is partly to maximise profit and partly to satisfy consumer demand for lean meat. Over the last fifteen years these selection techniques have resulted in genotypes with improved lean content, and an improved FCR of between 1 and 2% per annum (Webb 1989). Unfortunately, the selection of pigs to maximise lean meat production has resulted in reduced appetite and consequently, reduced food intake (Webb, 1989); Cole and Chadd 1989; Hill and Sainsbury, 1995; English *et al.* 1988). It has been suggested that genotypes with high lean and low carcass fat content are unable to maximise their lean tissue growth because of limited appetite (Campbell and Taverner 1988; Rao and McCracken 1992). However,

Urquhart, McEvoy and McCracken (1993) cast doubt on the hypothesis that appetite is limiting protein deposition in pigs of high genetic potential. They studied the effect of increasing food intake above the appetite limit by use of intragastric feeding and concluded that high genetic potential boars fed *ad libitum* were already achieving maximum rates of protein deposition. Although there appears to be some debate as to whether or not pigs of high genetic potential have reached their appetite ceilings with regard to lean deposition (Urquhart *et al.* 1993) there is no doubt that improved genotypes have an enormous genetic potential for growth which has not been fully exploited (MAFF 1987; Bolduan, *et al.* 1988; Pluske, *et al.* 1995).

The post natal piglet generally grows at 180 - 240 g d^{-1} between birth and weaning at 3 - 4 weeks of age (Pluske *et al.* 1995) and it has been reported by Harrell *et al.* (1993) that a growth rate of 300 g d^{-1} for a 5.0 kg piglet would represent a 6% increase in bodyweight per day. At 5 to 5.5 kg the piglet should be growing by between 250 and 350 g d^{-1}. Targets for the growth of piglets from birth to six weeks are presented in table 1.27. The MLC (1996) have reported that, in 1995, the top third of herds were capable of producing weaner piglets (average weight 6.4 kg) that would grow at 475 g d^{-1} with a FCR of 1.73 over a weight range of 6.5 - 36.7 kg, (Table 1.28).

Table 1.27 Target for growth performance of piglets from birth to weaning at different ages

Age (wks)	Liveweight (kg)	Target daily gain (g)
Birth	1.4	165
1	2.6	205
2	4.0	270
3	6.0	350
4	8.5	380
5	11.2	420
6	14.14	-

After (Hill and Sainsbury 1995).

Table 1.28 Overall rearing herd results for weaner pigs for the year ended September 1995.

Pig Performance	Bottom third	Average	Top third
Weight of pigs at start (kg)	6.5	6.5	6.4
Weight of pigs produced (kg)	31.0	34.9	36.7
Mortality (%)	3.3	2.7	2.4
Feed conversion ratio	1.98	1.79	1.73
Daily gain (g)	407	451	475
Feed cost per pig reared (£)	11.33	10.86	10.46

After (MLC 1996)

During the first week of post-natal life the energy contained in the body of a piglet increases by a factor of between four and five (Okai *et al.* 1977 cited by Pluske *et al.* (1995). Fowler and Gill (1989) have calculated that for the piglet to grow at this rate then it must consume metabolizable energy (ME) at the rate of four times its maintenance requirement. At 3 weeks of age the normal piglet (approximately 5.6 kg) requires about 7.8 Megajoules (MJ) digestible energy (DE) to sustain a live-weight growth rate of $280gd^{-1}$. This means that the piglet on a typical starter diet, would have to consume 475 g of food d^{-1} (*circa* 86% DM), assuming an energy value of 16.5 MJ DE kg^{-1} of food, if it were to maintain its pre-weaning growth rate. However, intakes of 475 g d^{-1} are rarely achieved (Fowler and Gill 1989).

Although the young piglet has an impressive growth rate when suckling the sow, weaning at 3 - 4 weeks causes an abrupt change which results in reduced voluntary food intake (VFI) and growth check (Leibbrandt, Ewan, Speer and Zimmerman 1975; Seve 1982; Fenton, Roerhig, Mahan and Corley 1985; Bolduan *et al.* 1988; Fowler and Gill 1989). Growth rate can be reduced to under 50 g d^{-1} in the first 24 hours post weaning and newly weaned pigs can fail to consume sufficient food for maintenance during the first 3 days post weaning (Hill and Sainsbury 1995) (Table 1.29).

Table 1.29 Growth performance of pigs weaned at 2, 3 or 4 weeks of age.

Age at weaning (wk)	Weeks post weaning 1	2	3	4
		Average daily gain (g)		
2	-38.3	124.0[a]	287.9[b]	359.4[a]
3	-24.8	233.5	394.8	516.9
4	3.4	306.8	492.0	564.1
		Average daily feed intake (g)		
2	95.0	213.8	363.8[c]	474.8[c]
3	138.1	315.1	488.4	641.9
4	154.2	409.6	597.8	762.4

[a] Significance ($P<0.01$) week effect for all ages;
[b] Significance ($P<0.05$) age effect within week;
[c] Significance ($P<.0.01$) age effect within week.
Adapted from (Leibbrandt *et al.* 1975).

There have been a large number of experiments conducted with piglets weaned shortly after birth (weaned < 21 days), and these have shown that when healthy pigs are fed *ad libitum* and provided with liquid diets of milk composition, there was generally an increased growth rate in contrast to piglets suckled by the sow under commercial conditions (Lecce 1975; Braude and Newport 1977; Bark, Crenshaw and Leibrandt 1986; Harrell *et al.* 1993). It has been suggested Braude, Mitchell, Newport and Porter (1970) that early weaning (weaned < 21 days) could have the following advantages: 1) reduced mortality through control of environment, 2) improvement in their growth rate by making available more nourishment than that supplied by the sow, 3) elimination of lactation and shortening of the reproductive cycle of the sow, thus increasing her productivity, 4) increased profit. Braude and Newport (1977) weaned piglets at 2 days of age and compared the same diet (730g kg^{-1} dried skim-milk powder and 230 g kg^{-1} soyabean oil) fed *ad libitum* either in liquid (20% dry matter) or pelleted form. Their results showed that piglets offered the liquid form grew faster than those offered the pelleted form of the diet, (281 g d^{-1} and 157 g d^{-1}

respectively), between 7 and 28 days of age. They concluded that the growth potential of piglets was only fully realized when they were offered a liquid diet.

1.12 Voluntary food intake of the pig

1.12.1 The significance of voluntary food intake (VFI).

The main purpose of animal production is to supply high-quality food for humans whilst the principal goal of animal scientists is to improve the efficiency of animal production which can in turn meet this demand (Beitz 1985). Animals need food for maintenance, repair, growth and reproduction (Schmidt-Nielsen 1990). The more food an animal consumes each day, the greater will be the opportunity for increasing daily production (Beitz 1985). The significance of VFI is that if food intake is too low then the rate of production is likely to be decreased, thereby increasing the requirements for maintenance and reducing the efficiency of food conversion (Forbes 1995). It is essential that VFI is maintained, especially in the newly weaned piglet, because it has such an important effect on the morphology of the gut.

1.12.2 Definitions of VFI

Forbes (1995) defined the VFI of an animal as being

> 'the weight eaten by an animal or group of animals during a given period of time during which they have free access to food'.

Cole, Hardy and Lewis (1972) described VFI as reflecting the needs of the pig in respect of its maintenance requirement and its productive requirements as represented by liveweight gain. Therefore, the amount of food consumed by the pig represents a balance between the needs of the pig and the ability of the food to meet those needs (Cole and Chadd 1989).

1.12.3 The regulation of VFI

VFI is regulated by the central nervous system (CNS) through a series of neural and endocrine interactions (Whittemore 1993). The controlling mechanisms which regulate feed intake are linked with a regulator of body energy content, thereby maintaining a balance of energy input and output under normal feeding conditions (NRC 1987). The hypothalamus includes centres that regulate appetite, body temperature and other autonomic functions (Procter 1982) and is thought to be involved in the regulation of energy in the body. Peripheral factors such as taste, smell, gastric distension, osmoreception, hepatic chemoreception, metabolites, hormones and temperature all have an influence on the mechanisms which regulate VFI. Metabolic processes influence, coordinate and integrate peripheral factors with neural elements and feeding either commences, continues or ceases (Sullivan and Cheng 1978). The mechanisms which regulate VFI have been previously reviewed (Anand 1961; Baile 1971; Sullivan and Cheng 1978; Russek 1981; Baldwin 1985; Fowler 1985; Forbes 1995) and the factors which affect VFI by (ARC 1981; Baile, Della-Fera and McLaughlin 1983; Fowler 1985; Henry 1985; Rayner and Gregory 1989) and the (NRC 1987).

1.12.4 Metabolic regulation of VFI

Three main theories have arisen to explain how the CNS and the hypothalamus are influenced with respect to the control of VFI. They are the glucostatic theory (Mayer 1955), the lipostatic theory of Kennedy (1953) and the thermostatic theory (Brobeck 1948). The glucostatic theory of Mayer (1955) has been proposed for short term control of VFI based on the fact that the satiety centre of the hypothalamus contains glucoreceptors (probably located in the ventromedial hypothalamus) sensitive to the concentration of glucose present in the blood (Anand 1961; Sullivan and Cheng 1978). Mayer (1955) observed fluctuations in blood glucose concentration in synchrony with meals and proposed

that the animal attempted to maintain a relatively constant level of glucose in the blood by a central nervous monitoring system. He suggested that increased blood glucose levels reduced food intake in rats. As glucose was utilised, arteriovenous differences in blood glucose levels were detected by glucoreceptors and feed intake was increased.

Research conducted by Bulato and Carlson (1924) cited by (Cole and Chadd 1989) reported a relationship between gastric hunger contractions and blood glucose level, but this was contradicted by Mulinos (1933) cited by (Cole and Chadd 1989). More recent evidence has shown that the liver may also be sensitive to glucose. In a review, Russek (1981) concluded that hepatic receptors were most important in determining hunger and satiety. However, there is still controversy as to the importance of the liver in the control of food intake under physiological conditions (Forbes 1995). The evidence for or against the glucostatic theory is inconclusive and it has been suggested by that the control of intake may depend on the whole complex of metabolites in the blood stream rather than on glucose alone Kennedy (1953) cited by Anand (1961).

Research has been conducted on the regulatory controls which involve sensitivity to metabolites and circulating hormones in rats (Gibbs, Fauser, Rowe, Rolls, Rolls, Maddison 1979) reviewed by Baile *et al.* (1983). It was proposed by Mellinkoff (1957) that concentrations of free amino acids in the body fluids may act as signals to regulate VFI, although this was contradicted by (Anand 1961). In the two decades there has been a considerable interest in the role of regulatory peptides, in particular the polypeptide gut hormone Cholecystokinnin (CCK), in the control of VFI (Sullivan and Cheng 1978; Baile *et al.* 1983; NRC 1987; Forbes 1995). CCK, an intestinal and brain hormone, appears to act as a satiety agent (NRC 1987).

For long term control of VFI the lipostatic theory of appetite control has been proposed, and states that the hypothalamus is sensitive to blood metabolites which in turn are influenced by fat mobilization (Kennedy 1953) cited by Anand (1961). Forbes (1995) stated that

> 'this 'long-term' signal must be integrated with the various 'short-term' signals in order that the sum total of the food eaten at a series of meals is appropriate to the animal's long-term requirements'.

Animals have a relatively constant weight and any deviation from this weight results in loss or gain of fat (Cole *et al.* 1972; Baile 1971). It was concluded by Mayer (1955) that

> 'the long-term regulation of body weight would be based on the fact that animals will mobilize spontaneously, each day, a quantity of fat proportional to, or at least increasing with, the total fat content'.

Cole *et al.* (1972) demonstrated that growing pigs which had undergone a period of feed restriction had depleted fat reserves and higher VFI on re-alimentation than those pigs which had been fed *ad libitum* throughout the trial period. However, the restoration of fat level did not appear to result in an immediate reduction in feed intake. The lipostatic theory has been extensively reviewed by Baile (1971) who concluded that the theory of lipostatis is inconclusive because the proposed communication mechanisms between satiety and adipose tissue have not yet been defined.

The thermostatic theory is based on the hypotheses that the heat of metabolism is sensed by the CNS and this regulates VFI. Brobeck (1948) believed that the important factor in regulation of food intake was not its energy value, but rather the amount of extra heat released in its assimilation. This extra heat, called the specific dynamic action, signals the hypothalamic mechanisms and thus adjusts the total quantity of food eaten (Anand 1961). Cole and Chadd (1989) stated that

> 'no direct physiological or anatomical links between food intake and temperature regulation have yet been identified'.

Temperature does have direct effects on VFI (Rinaldo and Le Dividich 1991). If pigs are housed in a hot environment they reduce their food intake in an effort to reduce the amount of body heat produced *via* digestive and metabolic processes (Schenck, Stahly and Cromwell 1992).

Cole and Chadd (1989) suggested that these three major hypotheses which have been proposed as regulators of VFI, and are based largely on monitoring glucose utilization, heat production or fat depots are all direct or indirect measures of the energy content of the major nutrients. The "energostatic" theory hypothesized that feeding was regulated by the metabolic energy status of the animal (Sullivan and Cheng 1978). Adolph (1947) observed that if food was diluted with inert material, animals quickly adjusted for the decreased caloric content per unit volume by increasing their food intake and he concluded that the energy value of the food determined VFI.

It is generally considered that the pig regulates VFI to maintain a constant daily intake of energy (Cole *et al.* 1972; NRC 1987; Whittemore 1993; Forbes 1995). Energy is the most essential constituent of the diet needed for essential body processes. Therefore, the energy concentration in the feed and the daily energy requirements of the pig will determine the weight of feed required (MAFF 1982a). As the energy density of the diet increases, VFI decreases and *vice versa*, in order to maintain constant energy intake (NRC 1987). However, when very high or very low nutrient density diets are fed, pigs are unable to compensate completely for variations in energy density (Cole *et al.* 1972; Forbes 1995). The NRC (1987) have reviewed research on the affect of varying energy content of diets fed to pigs on VFI, and concluded from the studies conducted with young pigs, (weighing on average from 5 to 30 kg), daily (DE) intake was relatively stable with diets containing 13.8 MJ kg^{-1} to 15.06 MJ kg^{-1}.

When the energy density of the diet was less than 13.8 MJ kg^{-1}, young pigs were unable to maintain daily DE intake, and daily DE intake decreased by 5.80 MJ d^{-1} as energy density decreased 4.18 MJ kg^{-1}. When the diets contained more than 15.06 MJ kg^{-1}, young pigs overconsumed energy, and DE intake increased at a rate of .765 MJ d^{-1} for every increase in energy density 4.18 MJ kg^{-1}. Pre-weaned piglets weighing between 5 and 5.5 kg should be capable of growing at 250 - 350 g d^{-1}. However, in the first 24 hours post weaning this can be reduced abruptly to 50 g d^{-1} (Hill and Sainsbury, 1995). As young pigs up to 20 - 25 kg liveweight have relatively small appetites MAFF (1982a) recommended that they should be given a high energy feed (DE = 13.7 MJ kg^{-1}) *ad libitum*, regardless of age at weaning, if they are to obtain maximum liveweight gain. The amount of feed that piglets of different weights would need to consume if the energy value of the diet varies from 13 to 14 MJ ME kg^{-1} diet. (Whittemore 1993) suggested that VFI was equal to 4 times maintenance, therefore, using this equation it is possible to calculate the maximum VFI that could be anticipated for weaned pigs of different weights and for diets of different energy content (Table 1.30).

Table 1.30 Naive calculation of maximum potential feed intake of weaned piglets of different weights fed diets of different energy content.

Liveweight (kg)	5	6	7
$W^{0.63*}$	2.76	3.09	3.41
M (MJ ME d^{-1})†	1.987	2.225	2.455
VFI (M x 4) (MJ ME d^{-1})	7.948	8.900	9.82
Energy value of diet	Indicated maximum food intake in grams		
13 MJ ME kg^{-1}	611	685	755
14 MJ ME kg^{-1}	568	636	701

* 0.72 MJ ME kg^{-1} $W^{0.063}$, metabolic body weight (Equation 13.5) (Whittemore 1993); † Voluntary food intake (Equation 13.4) (Whittemore 1993).

(Cole, Duckworth, Holmes and Cutherbertson 1968b) hypothesised that physiological control of VFI by pigs, was affected by physical limitation with diets of low density, and lack of gut fill with diets of high density, but they did not give energy values outside which normal responses could not be expected to operate. Whittemore (1993) has stated that

> 'appetite is limited by the physical capacity of the gut and rate of digesta throughput and that this will be related to body size'.

There is some indirect evidence that gastric distension limits intake in baby pigs. Wangsness and Soroka (1978) fed piglets a liquid diet with decreasing energy content and as the energy content of the diet was reduced the piglets attempted to compensate by increasing VFI but could not maintain energy intake and weight gain at the greatest dilution. They concluded that complete dietary compensation was prevented by gastric distension. Yang, Howard and Macfarlane (1981) also suggested that abdominal fill (*i.e.* the sum of water and food intake) may be important in the regulation of VFI. In their study pigs of 30 kg were used and it was concluded that pigs of this weight had a limit on their daily intake of total dry matter and water equal to 19% of their liveweight. Below this limit the pig would consume food as a first requirement and limit water intake to a minimum level. Barber (1992) conducted similar experiments with older pigs, weighing between 30 - 60 kg, and concluded that volumetric intake was a limiting factor on dry matter intake, and that the total volumetric intake was approximately 12% of the liveweight.

Other research workers have hypothesized that VFI is influenced by sensations from the digestive tract associated with eating, swallowing and the presence of food in the stomach and intestines (Janowitz and Grossman 1949; Share, Martyniuk and Grossman 1952; Rayner and Gregory 1989). Anand (1961) reviewed experiments to elucidate the role of the GI tract in producing satiety and concluded that gastric distension and sensations from the GI tract were important factors. From their studies with pigs, Rayner and Gregory (1989)

suggested that gastric emptying may also be implicated in the 'short-term' control of VFI. In their experiment the amount of fat infused into the stomach of the pig was compensated almost exactly by reduced VFI.

Anand (1961) also described several experiments which suggested that there was a correlation between the regulation of food intake and water intake. Strominger (1947) cited by (Anand 1961) observed in rats that, within limits, the higher the water concentration of the diet the greater the food intake. It has been suggested that "osmoreceptors" (cells which respond to changes in water concentration in body fluids) (Roberts 1986) are involved in regulation of food intake (Anand 1961). The suggestion that osmotic pressure may influence VFI was explored by Lepkovsky, Fleming, Nagumo and Dimick (1957) who observed that when rats were fed without water they ate less food than rats fed with water. They concluded that rats regulate their food intake so that it matches the amount of water that they can mobilize from their own tissues, thereby maintaining the proper water:food ratio in the gastric contents. Anand (1961) stated that

> 'for short periods of time, mobilizable water from the animals tissues may regulate food intake, with little regard to the total water balance of the body or the excretory load upon the kidneys.

Chew (1965) cited by (Yang *et al.* 1981) reviewed the relationship between water intake and food intake in animals and suggested that there is a close and positive relationship between the amount of food eaten and water ingested. However, Yang *et al.* (1981) concluded from their experiments on growing pigs that there was no positive correlation between food and water intake.

The importance of these latter experiments is that they indicate that dry matter intake should not be viewed in isolation. The bulk and water content of the diet and the demand for water that the dry matter creates are significant factors affecting VFI.

1.12.5 Factors which affect the VFI of the pig.

There are many factors in the diet which affect the controlling mechanisms of VFI, such as, deficiencies or excesses of nutrients, antibiotics, inadequate water supply, anti-nutritional factors, flavours and feed processing (Henry 1985; NRC 1987; Brooks and Carpenter 1990; Fraser, Patience, Phillips and McLeese 1993; McCracken and Kelly 1993; Whittemore 1993; Brooks 1994; Forbes 1995). Other factors which also have an affect on VFI are environmental conditions, inadequate management, insufficient space and the behaviour of the piglets themselves (Whittemore 1993). The affect of these factors on VFI is important in the management of pigs if reasonable targets for performance are to be achieved. Some of these factors will be discussed in more detail with particular reference to the needs of the newly weaned piglet.

1.12.6 Environmental conditions which affect the VFI of the pig.

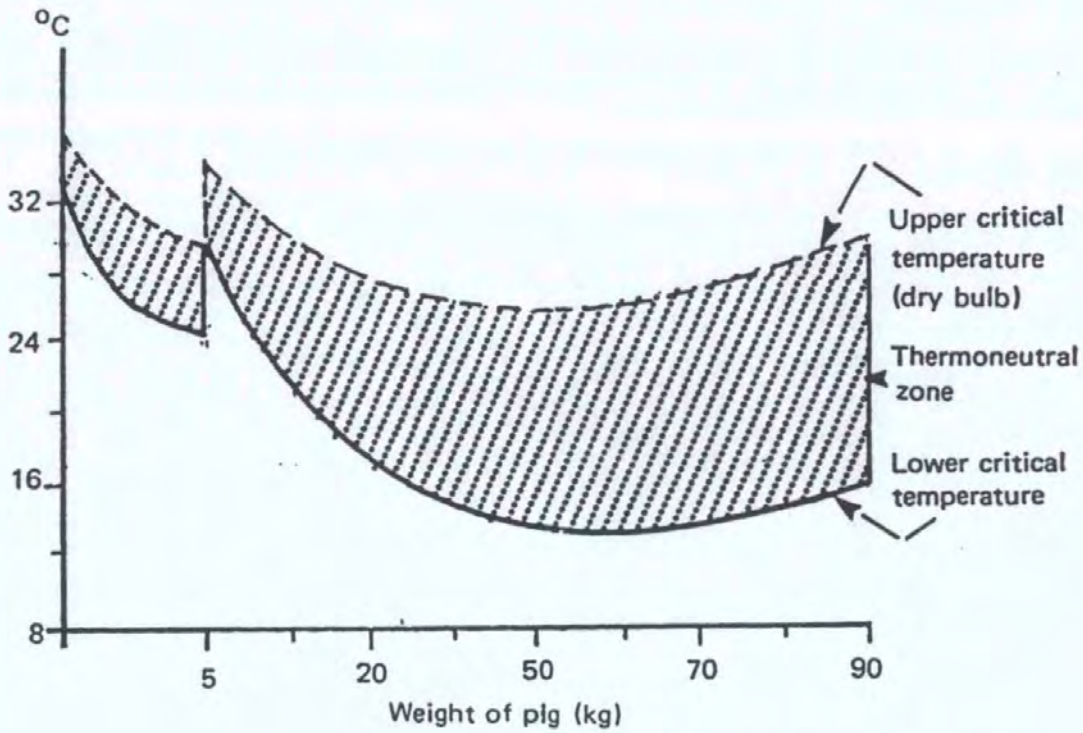
Over the past few years considerable attention has been paid to the climatic requirements of the newly weaned pig (Le Dividich and Herpin 1994). A major component of the stress suffered by piglets can be of thermal origin and temperature is probably the most significant component of the environment that effects VFI in piglets (NRC 1987; Fowler and Gill 1989). The piglet is poorly endowed with hair and subcutaneous fat and has thin skin (Hill and Sainsbury 1995). In addition it has a high surface area to volume ratio which results in a high sensible heat loss and hence maintenance requirement is higher in the young pig. Food intake is low and activity high in the newly weaned piglet (McCracken and Caldwell 1980). This may result in a negative energy balance during a period 4 - 6 days following weaning (Le Dividich and Herpin 1994). As the piglet still requires energy for maintenance, activity and protein synthesis, body fat reserves are depleted. This loss is inversely related to environmental temperature (Le Dividich, Vermorel, Noblet, Bouvier and Aumaitre 1980). The initial fat content of the piglet is not

regained until approximately 4 to 6 weeks post weaning (Seve 1982). The loss of body fat reduces body thermal insulation (Le Dividich *et al.* 1980; Fenton, *et al.* 1985) increasing the rate of heat loss and increasing the heat demand (Le Dividich *et al.* 1994).

The pig has a deep body temperature of 39°C (MAFF 1982b), and is most comfortable and productive when it is kept in a thermal environment consistent with its zone of minimum metabolism (its thermoneutral zone) (English, *et al.* 1988). The upper and lower limits of this zone are designated the upper critical temperature (UCT) and the lower critical temperature (LCT) (MAFF 1982b) (Figure 1.7). The heat which is produced in the pigs body is a direct result of metabolism and is proportional to body mass. Heat is lost as a result of conduction, convection, evaporation and radiation and is proportional to surface area exposed. Between the UCT, and LCT, energy is not required by the pig to maintain body temperature, and VFI is relatively stable (NRC 1987). Keeping piglets within this thermoneutral zone should improve efficiency and productivity (English *et al.* 1988).

The energy contained in the feed eaten will govern the rate of heat produced whilst the size of the pig, its behaviour (posture) and it's surroundings will influence the rate of heat loss. When feed intake is restricted air temperature requirements increase, consequently weaned piglets have a much higher LCT due to their high surface area : volume ratio and comparatively low VFI. Ambient temperatures above the piglet's UCT result in reduced VFI because the pig consumes extra water to assist in dissipating metabolic heat and this increased water intake limits feed intake (Brooks and Carpenter 1990). McCracken and Caldwell (1980) suggested that LCT of the piglet will be in the region of 26 - 28°C during the first week post weaning, when the average level of VFI is lower than the metabolizable energy for maintenance.

Figure 1.7 Example of a thermoneutral zone pattern.



After Ministry of Agriculture, Fisheries and Food (1982)

Le Dividich (1981) agreed with this temperature range and suggested that this could be reduced by 2 to 3°C per week until the temperature to be maintained in the finishing house had been reached. From studies conducted with weaner pigs (14 or 28 days of age), kept on perforated floors, in still air conditions McCracken and Kelly (1993) concluded that an air temperature of 26°C is desirable in the first week post weaning. They recommended a reduction of 1°C d^{-1}, to 20°C, as long as piglets were kept in draught free conditions and were healthy.

When piglets are kept at temperatures above their UCT, performance will be depressed and if those temperatures reach extremes, heat stress will drastically reduce VFI (NRC 1987). Alternatively, when piglets are kept in temperatures below their LCT piglets become susceptible to disease (NRC 1987), have poor feed conversion efficiency and may develop scours (MAFF 1982b). Le Dividich and Herpin (1994) suggest that piglets can overcome sub-optimal temperatures by increasing VFI but this is not true for newly weaned piglets which are already stressed (Fowler *et al.* 1989). English *et al.* (1988) stated that

> 'for each 1°C below LCT a pig of 10 kg will require an extra 5 g of food daily to compensate'.

Fluctuating temperatures can also have a detrimental affect on weaned piglets. Le Dividich (1981) observed that there was a greater incidence of post weaning diarrhoea in piglets kept in a continuously (hourly) fluctuating temperature 23.5 ± 3°C compared to those kept in a constant environment of 23.5 ± 0.5°C, and that diarrhoea was highest in the first week post weaning.

Some researchers have investigated the effect on the performance of newly weaned piglets of reducing nocturnal temperatures (McCracken and Caldwell 1980; Brumm and Shelton

1991). Pigs exhibit circadian rhythms with respect to their metabolic activities, which are lower during the night. If their activity is lower at night then in theory heat loss should be less and nocturnal temperatures could be reduced to save on cost. Brumm and Shelton (1991) reported that a 4 to 9°C reduction in nocturnal temperature did not affect the VFI of 3 - 4 week old weaners but did increase the severity of scouring. Rinaldo and Le Dividich (1991) reported that within a 12 - 20 kg liveweight range, pigs which were subjected to an 8°C reduction in nocturnal temperatures had an increased VFI compared with those maintained at 22°C (Table 1.31).

It would appear that if the productivity of weaner piglets is to be maximised, the thermal environment must be accurately controlled in order to maintain VFI, and at the nutrient requirements of the piglets have to be met within the pig's intake capacity. Some researchers have produced mathematical models to predict the intake of pigs under different conditions of environmental temperatures (NRC 1987).

Table 1.31 Effect of a reduction of 8°C in nocturnal temperature on the performance of weaned piglets raised in groups[a]

Treatment	Constant temperature (22°C)	Reduced nocturnal temperature (22-14°C)
Growth rate, g d^{-1}	626	643
Food intake, g d^{-1}	1070	1210
Food conversion ratio	1.71	1.88

[a] Piglets were weaned between 3 and 4 weeks of age, initial bodyweight averaged 12.3 kg
After Rinaldo and Le Dividich (1991)

Rinaldo and Le Dividich (1991), proposed the following relationship between temperature and intake (Equation 1.1)

$$\text{Intake (g d}^{-1}) = 1163 + 16.8T - 0.8T^2$$

where T = environmental temperature. (Equation 1.1)

Forbes (1995) interpreted this equation as indicating that, for a 20 kg pig, there is an increase of 1.5% in VFI for every degree decrease in environmental temperature within the range of 15 to 25°C.

The temperature of the environment can be modified by physical factors, such as moisture content and air speed and these factors can interact to influence feed intake of pigs (NRC 1987). Management of ventilation in piggeries is important in that it is needed to reduce humidity, remove noxious gases and pathogens and to influence the control of temperature (Le Dividich and Herpin 1994). If piglets are kept in draughty conditions this can also have adverse affects on their health, for example, an increase in coughing, sneezing and diarrhoea (Scheepens, Tielen and Hessing 1991), and can reduce growth rates by 106 g d^{-1} within the first 10 days post weaning (Le Dividich and Herpin 1994). Hill and Sinsbury (1995) stated

> 'that if air speed is doubled, air temperature requirements rise by 12°C and this affects the comfort level of piglets'

as presented in table 1.32.

The number of pigs per pen and space allowance influences VFI and performance (Lindvall 1981; Kornegay and Notter 1984; Lindemann, Kornegay, Meldrum, Schurig and Gwazdauskas 1987; English *et al.* 1988). Lindemann *et al.* (1987) examined the effect of feeder space allowance on weaner pig performance and concluded that feeder space allowance had no effect on feed efficiency and was generally without affect on VFI and daily gain. This is at variance with English *et al.* (1988) who suggested that inadequate feeder space allowance does have a detrimental affect on VFI. Whittemore (1993) also considers that there is a significant influence of space allowance on feed intake and growth rate and suggested that for pigs weighing < 25 kg minimum feeder space of 0.15 m^2 per pig should be provided. The OJEC (1991) Council Directive (No L340/33) which has now

become a regulation, states that the unobstructed floor area for each weaner or rearing pig, reared in a group must be at least; 0.15m^2, 0.20m^2, 0.30m^2 ,0.40m^2, 0.55m^2, 0.65m^2 and 1.00m^2 for a pig of an average weight of 10, 10-20, 20-30, 30-50, 50-85, 85-110 and 110 kg respectively.

Table 1.32 The combined effects of ambient temperature and air movement on pig comfort.

Temperature	Air movement below 0.15 m s^{-1}	Air movement 0.15–0.25 m s^{-1}	Air movement 0.25–0.38 m s^{-1}
21°C	Pigs of all ages comfortable	Pigs of all ages comfortable	Young piglets uncomfortable (1–8 weeks)
18°C	Pigs below 1 week uncomfortable	Pigs below 5 weeks uncomfortable	Pigs below 12 weeks uncomfortable
15°C	Pigs below 10 days uncomfortable	Young piglets (approx. 1–3 weeks uncomfortable)	Pigs below 12 weeks uncomfortable
13°C	Pigs below 8 weeks uncomfortable	Pigs below 12 weeks uncomfortable	Pigs below 14 weeks uncomfortable
10°C	Pigs below 15 weeks uncomfortable	Pigs below approx. 16 weeks uncomfortable	Pigs below 16 weeks uncomfortable

m s^{-1} = metres per second
After (Hill and Sainsbury 1995).

1.12.7 Dietary factors which affect the VFI of the pig.

Although the energy density of the diet is a major factor affecting VFI, the concentrations of specific nutrients such as amino acids are also of great importance (Forbes 1995). Pigs can usually tolerate a mild over or under supply of nutrients in the diet but when excesses are more pronounced VFI is usually reduced (NRC 1987), for example very high or very low protein content of food can depress VFI in pigs (Cole and Chadd 1989; Forbes 1995).

If the protein:energy ratio of the diet is incorrect the performance of pigs will suffer because an excess of energy will lead to the deposition of fat instead of lean, and an under supply of energy will reduce deposition of both lean and fat.

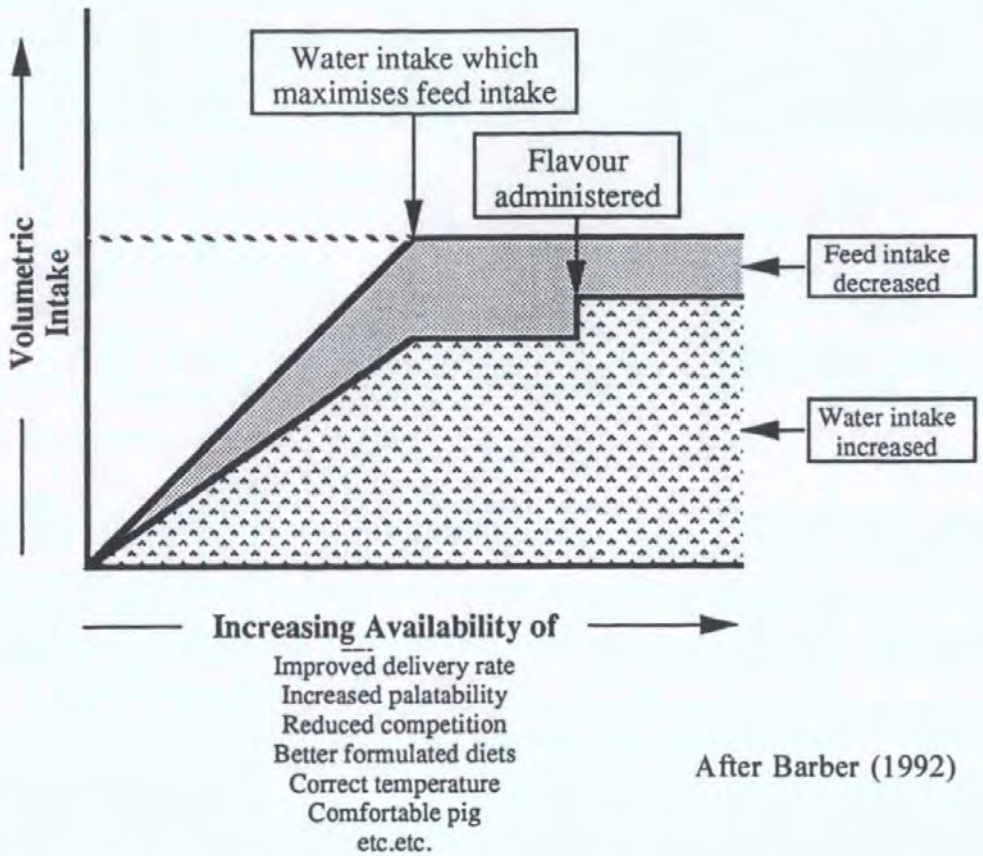
Protein in the diet is digested and absorbed as amino acids. It is important that the maximum rate of body protein deposition be achieved with as little wastage of the ingested amino acid as possible because an avoidable loss of amino acids is inherently wasteful biologically and excretion of nitrogen contributes to environmental pollution (Moughan 1991). Although over 200 amino acids have been isolated from biological materials, only 20 of these are commonly found as components of proteins (McDonald, *et al.* 1988), and nine are essential to the pig.

The amino acid requirements of pigs have been previously reviewed (NRC, 1988; ARC, 1981; Moughan 1991; D'Mello 1994) and Whittemore (1993). Where pigs are expected to achieve optimum performance the diet must contain adequate amounts of these essential amino acids (NRC 1988) because deficiencies of essential amino acids can depress VFI rapidly and severely (NRC 1987) and excesses can lead to a variety of negative syndromes that have been classified as toxicity, antagonism, and imbalance (NRC 1988; D'Mello 1994). This has led to the development of the concept of 'ideal protein', which has been reviewed by (D'Mello 1994; Whittemore 1993; ARC 1981; NRC 1988; Baker, Hahn and Chung 1993; Cole and Van-Lunen 1994). The concept stems from the supposition that there is some protein or mixture of proteins which supplies amino acids in exactly those proportions in which the animal requires them (Fuller and Chamberlain 1995). Pigs will consume feed to the first limiting amino acid and if the balance of amino acids in the diet are incorrect, *i.e.* one amino acid is supplied in excess, the pig will consume more water in order to excrete the nitrogen resulting from de-amination (Pfeiffer, Henkel, Verstegen

and Philipczyk 1995). The two major nutritional factors known to increase the water demand are: 1) the quantity and quality of protein in the diet and 2) the mineral content of the diet particularly the sodium and potassium levels (Brooks and Carpenter 1990). Pfeiffer *et al.* (1995) demonstrated that in growing pigs a higher daily protein intake increased water consumption ($P<0.05$), and increased the volume of daily urine excretion. This is because the pig has a limited ability to concentrate nitrogen in its urine. Thus, having extra nitrogen to excrete means that it has to increase water intake. If extra water has to be consumed then the increased volumetric fill of the pig may restrict dry matter intake and performance will suffer (Barber 1992). The more the amino acid supply deviates from 'ideal', the greater the demand for water (Brooks and Carpenter 1990). The effects of water and mineral balance on VFI have been extensively reviewed by Gill (1989) and Barber (1992). Barber (1992) summarised the affects of water consumption on VFI in a model (Figure 1.8). He demonstrated that as the availability of water increased VFI increased proportionally up to the maximum set by total volumetric intake. This relationship could be overridden by manipulating the palatability and hence consumption of water to such an extent that the increased water consumption reduced dry matter intake. Barber's work highlighted the importance of water consumption on VFI by demonstrating that the availability of water influences the amount of water the pigs consumes and this in turn affects VFI (Figure 1.9) and subsequent performance.

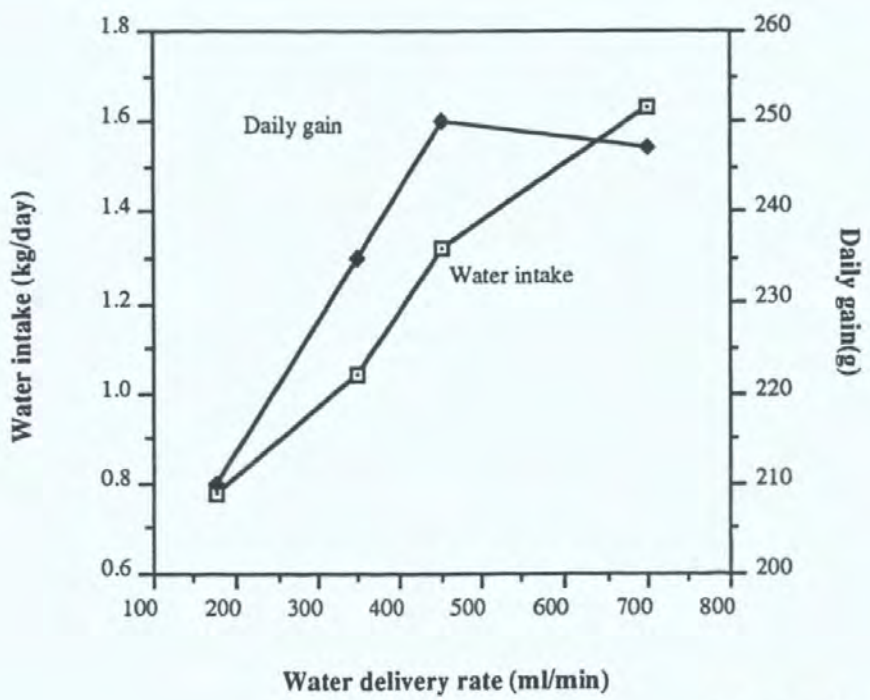
The palatability of the diet can affect the VFI of the pig. The term palatability is used to describe the degree of readiness with which a particular food is selected and eaten, and involves the senses of olfaction, touch and taste (McDonald *et al.* 1988). Palatability has been used to describe the sensory qualities of food, which include flavour, appearance, texture, temperature and taste (Lawrence, Appleby, Illius and MacLeod 1989).

Figure 1.8 Schematic model of the relationship between volumetric intake, water intake and feed intake in the pig.



After Barber (1992)

Figure 1.9 Effect of water delivery rate on water intake and growth of weaned pigs.



After Brooks (1994)

It has been reported that these food qualities influence VFI in animals, both positively and negatively, although there is some debate as to whether palatability can have a positive affect on VFI. Kyriazakis (1994) suggested that the effect of the sensory characteristics of the food on the rate of VFI are only transient and appear to have only an incentive value to the animal.

Pigs will readily eat cooked and flaked cereal, oils and fresh and dried milk and prefer sweet tasting feedstuffs. However, when ingredients such as meat-and-bone meal, some fish meals, rape-seed meal and cotton-seed meal are included in the diet, appetite may be depressed (Whittemore 1993). An adverse affect on intake would be experienced by pigs if they consumed foods which contained lectins (Kempen 1993), mycotoxins (Marguardt 1996), glucosinolates, tannins, saponins, sinapines, and other toxic anti-nutritional factors (Newby, *et al.* 1985; Fowler and Gill 1989).

1.12.8 Summary of the factors affecting voluntary food intake of newly weaned piglets.

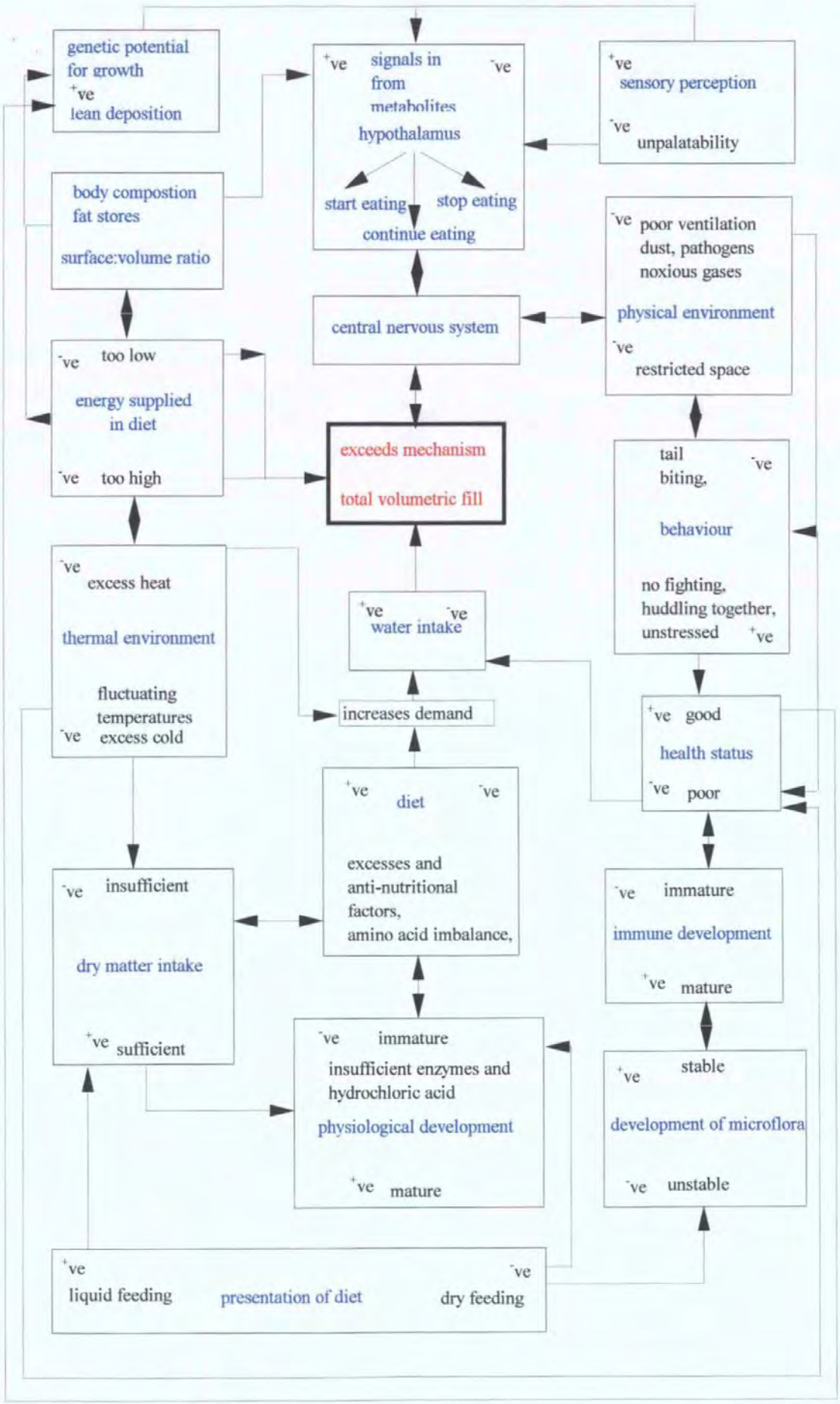
There is no one mechanism controlling food intake but rather a whole range of interactive mechanisms which act in concert and are in turn acted on by external and internal factors. Many experiments have been designed to examine specific factors involved in the control of VFI. Experiments in which only one parameter (*i.e.* temperature) is being changed inevitably suggest that a particular mechanism is predominant. However, the apparent influence of that factor may be a function of the experimental design rather than a true indication of its significance. The unidimensional approach taken in most studies may be the reason why there have been so many different and conflicting opinions about the factors controlling VFI. In reality a complex multifactorial control system exists which attempts to accommodate information coming in from a range of sources, to evaluate and coordinate these, and then react accordingly. A model has been constructed (Figure 1.10) which

attempts to illustrate the principal relationships between the regulating mechanisms and factors which influence the VFI of newly weaned piglets.

From the literature it is apparent that one of the major ways in which dietary excesses exert their effect on VFI is through increased water consumption and the consequent reduction of VFI. When piglets are weaned they are challenged with diets which may contain excesses of nutrients or anti-nutritional factors. They are also challenged by the physical and microbial environment, all of which will result in an increased consumption of water and a reduction in VFI. In an 'ideal' situation all the mechanisms controlling VFI should work together to regulate VFI satisfactorily. However, at weaning behavioral, physical and dietary changes may have an overriding effect on the pig and result in VFI being restricted.

In order to maximise VFI in newly weaned piglets the diet must be constructed from ingredients which are readily digestible, provide the correct proportions of essential nutrients and are free from anti-nutritional factors. In addition the composition and presentation of the diet must maintain palatability. Water must be freely available at all times, and the thermal and physical environment controlled to meet the requirements of the piglet.

Figure 1.10 Some relationships between the regulating mechanisms and factors influencing the voluntary feed intake of newly weaned piglets.



1.13 Rationale and objectives

Weaning is a crucial event in a piglet's life. The process of weaning presents the piglet with three major challenges. Firstly, it has to adapt to an abrupt change in diet. Secondly, it encounters a new environment, and thirdly, it has to establish it's position in a new social group. As a consequence of these challenges the piglet suffers from a disruption to it's nutrient intake and a change in dietary substrate. This can produce adverse effects on gut morphology and on the indigenous beneficial microflora present in the gastrointestinal tract of the piglet. At best this results in a short term disruption in growth, 'post weaning growth check', at worst this leads to enteric disease which has profound effects on gut health and may have long term adverse effects and may even cause death. Therefore, in order to succeed with the rearing of newly weaned pigs, the emphasis must be to establish a smooth transition from dependence on the sow to independence. As the piglet has been receiving a liquid diet of sow's milk pre-weaning it would appear logical to provide a liquid diet post weaning. Although attempts have been made to liquid feed weaners, these have generally not been commercially adopted. This is because of the problems associated with ensuring that the piglet was presented with a palatable, wholesome diet at regular intervals. The alternative approach, 'dry feeding', presents the nutritionist with many difficulties. For example, the problems of low feed intake and immaturity of the piglet's digestive system. This means that diets which are complex and expensive are needed to obtain satisfactory levels of performance. Two factors were instrumental in initiating the programme of work described here. First, the development of new equipment which facilitated the *ad libitum* liquid feeding of weaners. Secondly, the desire of commercial producers to reduce the cost of feeding weaners, by the incorporation of lower cost, liquid feed components in diets.

Therefore, the principal objectives of this study were;

- To assess the efficacy of a new, automated, *ad libitum*, feed delivery system for newly weaned piglets.
- To assess the affects of liquid feeding on the performance of newly weaned piglets.
- To explore the possibilities for reducing diet cost by using lower cost liquid components.

CHAPTER 2

GENERAL DESCRIPTION OF FACILITIES AND METHODS

2.1 Building design and features

The pigs were housed in an environmentally controlled, flatdeck weaner house, comprising 10 pens. The layout of the building is illustrated in figures 2.1 and 2.2 and plate 2.1. Each pen was 1.44 x 1.25 m in floor area giving a space allocation of 0.30 m^2 per pig when stocked at six pigs per pen. The pen floors were totally perforated (Cumfidek, Big Pig, Deeside, Clwyd) and beneath each pen was a stainless steel effluent tank which could be emptied *via* a valve outside the building.

The stainless steel effluent tanks were calibrated by adding 100 l of water to the empty tanks using a metered water hose. A measurement was taken of the depth of water using a metre ruler. A further 100 l of water was added to the tank and this figure was subtracted from the previous 100 l. The difference in measurements taken with the metre ruler was used as a calibration figure (Appendices Table A 1).

Each pen was provided with water from two low pressure drinkers (Arato 76, Bernard Partridge, Weeley Heath, Essex) positioned 20 cm above the floor and supplied from a low pressure break tank system (Zerho$_2$pipe, Carbro Consultants, Newton Abbot, Devon). The water delivery rate from the drinkers was adjusted to exceed 450 cm^3 min^{-1} as flow rates below this were shown to adversely affect the performance of weaner pigs (Barber 1992). Water intakes were recorded using turbine flow water meters (PSM-L, Kent Meters, Luton, Bedfordshire).

Figure 2.1 Schematic diagram of the internal layout of the flatdeck weaner house.

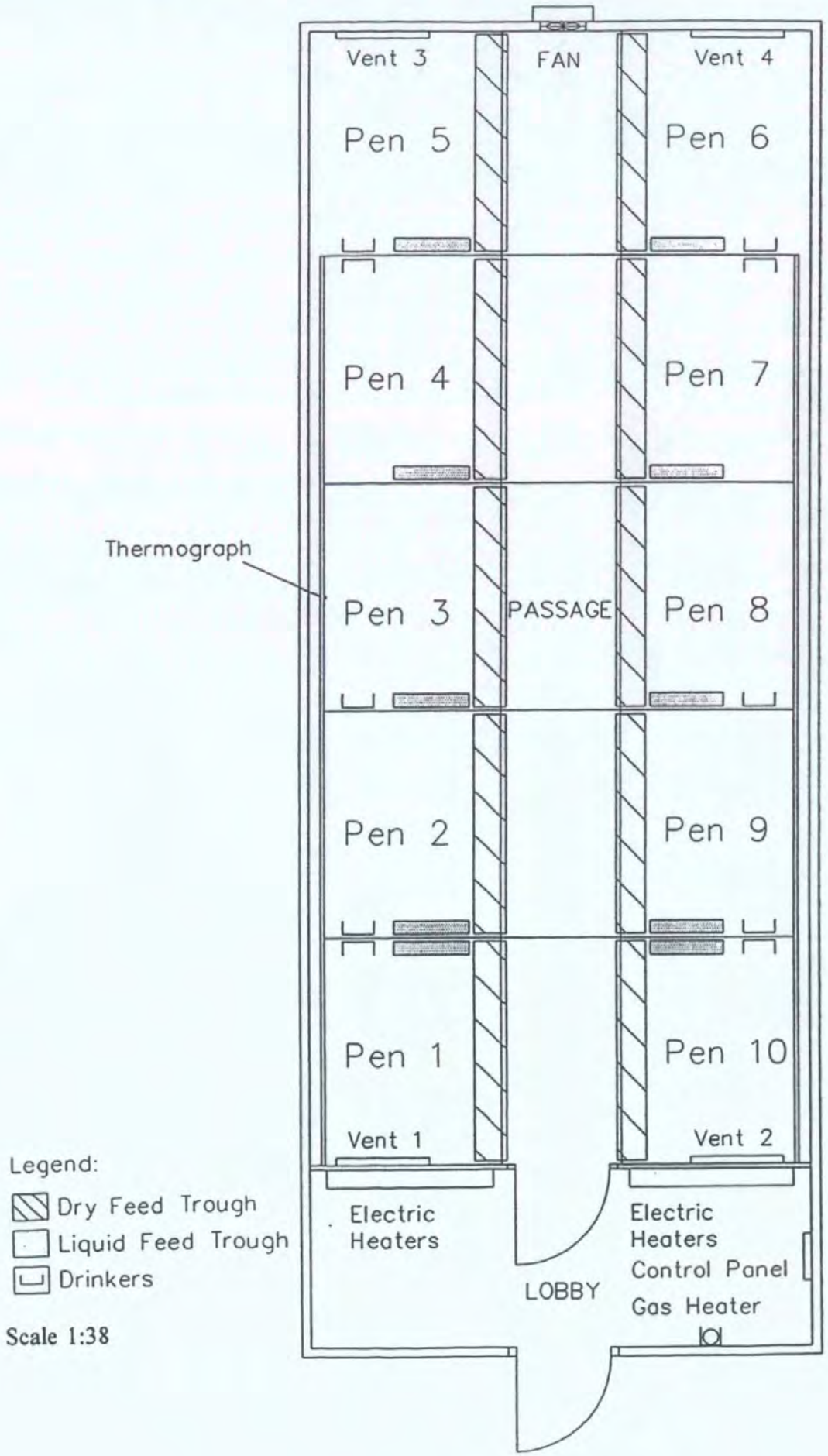
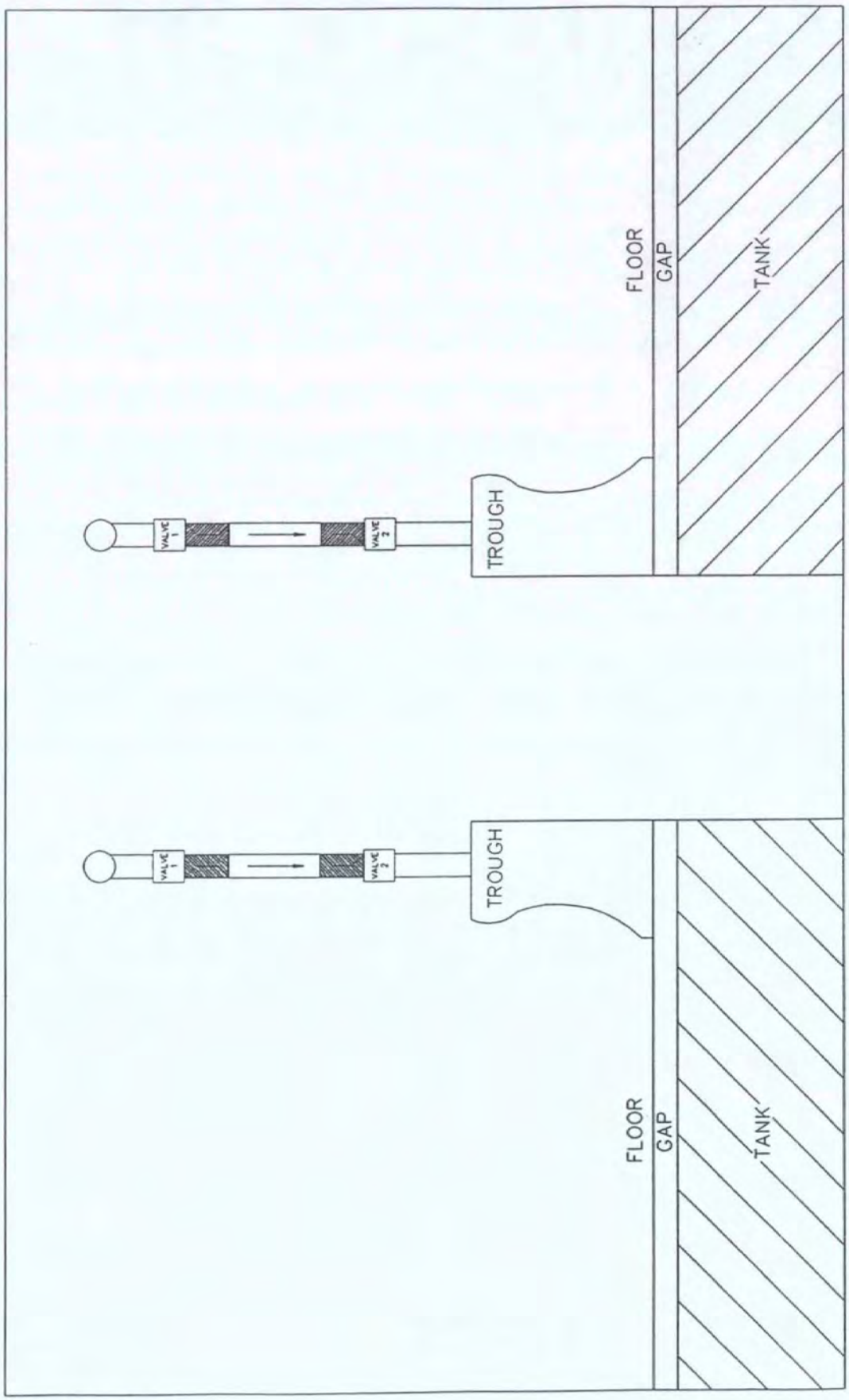


Figure 2.2 Cross section of the internal layout of the flatdeck weaner house.



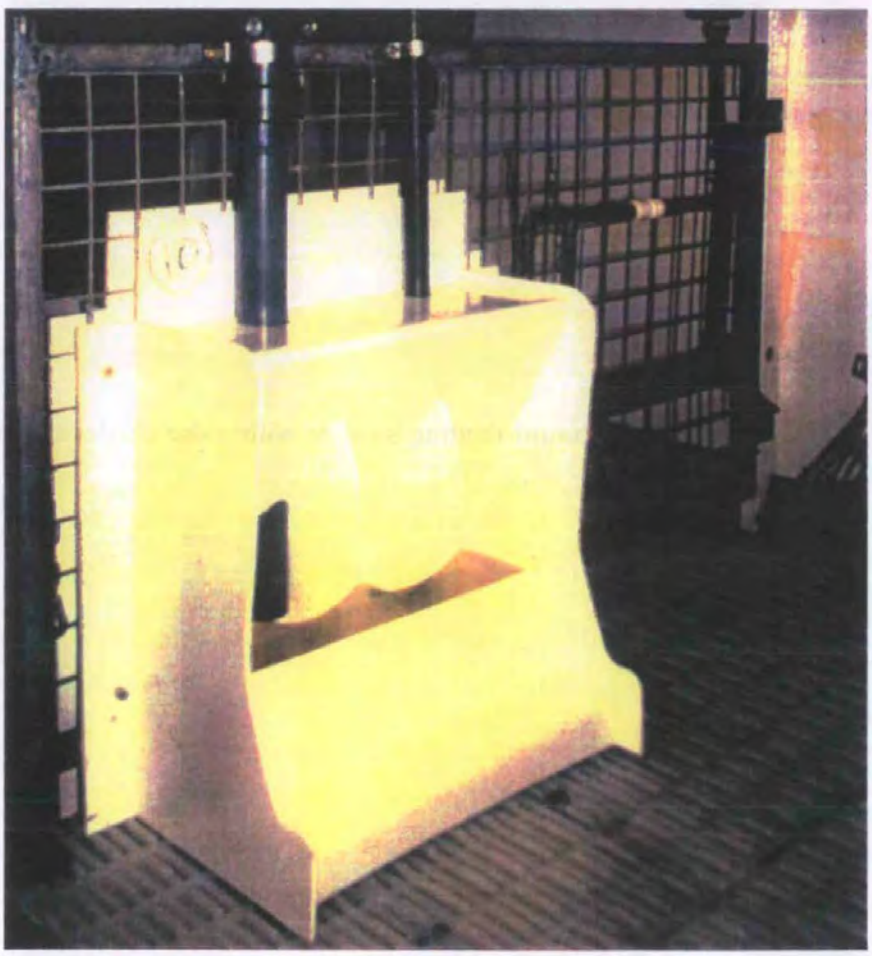
Scale 1:14

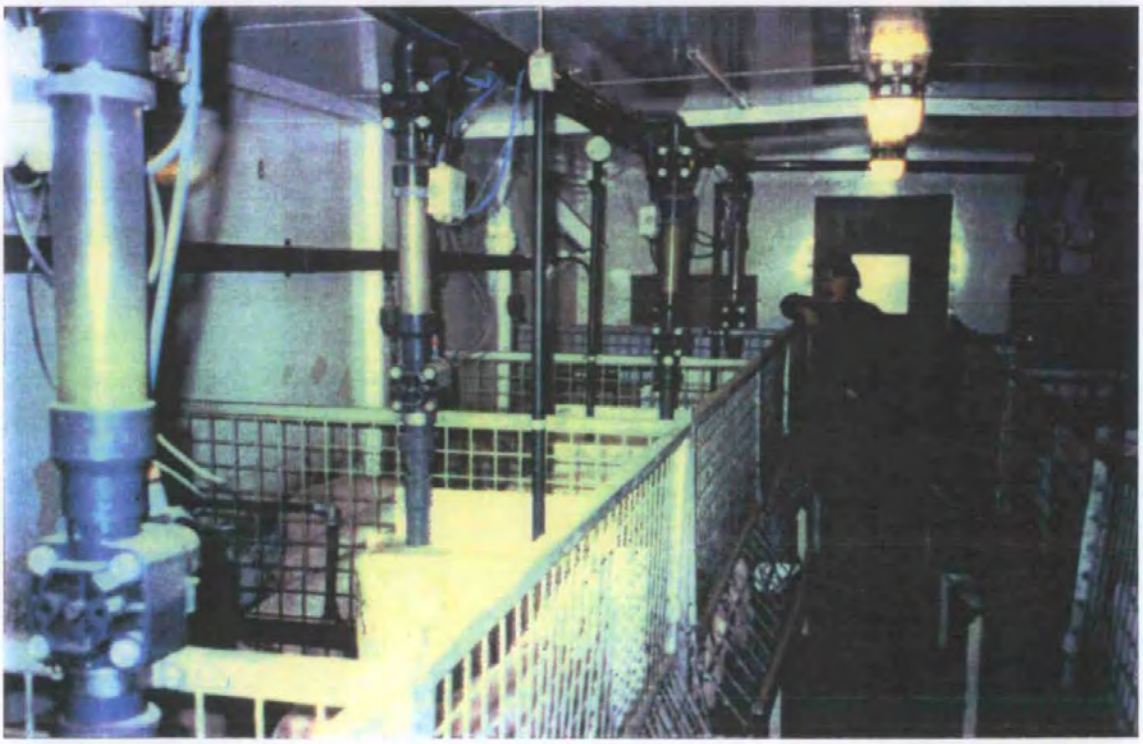
Pigs offered dry feed (**DF**) were fed in a feed trough 1.42 m in length which formed the front of the pen. This enabled all pigs in the pen to eat simultaneously if they desired. Troughs were emptied daily and residual feed weighed back. Any wet or fouled feed was removed, dried and weighed to estimate wastage. Daily additions of feed to the trough were recorded.

In Trial 1 pigs fed liquid diets (**LF**) were fed from a 40 x 20 cm stainless steel trough with no internal divisions and a capacity of 5 l. The trough was 10 cm deep and the front edge was 10 cm above floor level. In all subsequent trials pigs fed liquid diets were fed from a 44 x 10 cm plastic trough with two internal divisions dividing the trough into three equal compartments which had a capacity of 2 l (Plate 2.2). This latter design incorporated solid divisions between the feeding spaces that extended back to the shoulder of the pig when eating and a step in front of the feed trough on which the piglet had to stand to gain access to the feed trough. The front edge of the trough was 18 cm above floor level. The troughs were fixed to the shorter pen wall which formed the division between pens.

2.1.1 Feed system

The liquid feeding system (Hampshire Feeding Systems Ltd., New Milton, Hampshire) comprised a 450 l mixing tank, and a circulation system which enabled the liquid feed to be pumped around the building and returned to the mixing tank (Plate 2.3). Liquid feed was supplied to the feed trough through a vertical down pipe; the delivery being controlled by two pneumatically controlled valves. The two pneumatic valves were separated by a length of pipe which had a swept volume of one litre. The liquid feed dispensers were calibrated by operating the system with water and allowing the liquid feed dispenser valves to open dispensing the contents of the 1 litre aliquot into a beaker.





The quantity of water in the beaker was measured using a measuring cylinder. This process was repeated 3 times, and the mean value taken as the calibration figure (Appendices Table A 2). Opening the lower valve allowed the 1 litre aliquot of feed to be dispensed into the trough as illustrated in plate 2.4 and figure 2.3. The dosing mechanism was then replenished from the circulation system by closing the lower and opening the upper valve. Feed was circulated for a period of twenty minutes in every hour ensuring that the feed troughs never became empty. Liquid sensors in the troughs initiated replenishment of the troughs and this process together with recording of the liquid feed additions to each pen was automatic. The liquid feed system was duplicated in order that two treatments of liquid feed could be offered at the same time. Tank 1 supplied liquid feed system 1, which delivered liquid feed to pens 1, 3, 5, 7, & 9 and Tank 2, supplied liquid feed system 2, which in turn delivered liquid feed to pens 2, 4, 6, 8 & 10, (Figure 2.1).

2.1.2 Cleaning process

Cleaning of the liquid feed troughs was undertaken only if they became fouled, which was a rare occurrence. In such circumstances feed losses were estimated by drying and weighing the soiled feed. The liquid feed system was cleaned at the end of each feeding trial. This was done by draining the liquid feed tanks and liquid feed dispensers, followed by washing out the tanks with clean water. The tanks were then filled with clean water and the system operated to flush out the pipes and liquid feed dispensers. This process was repeated again using a solution of hypochlorite (mixed in the ratio of 100 mls of hypochlorite to 40 litres of water) followed by clean rinsing water. After flushing the system, the liquid feed and effluent tanks were drained of liquid and the building was pressure washed. The building was then allowed to dry for a period of three days.

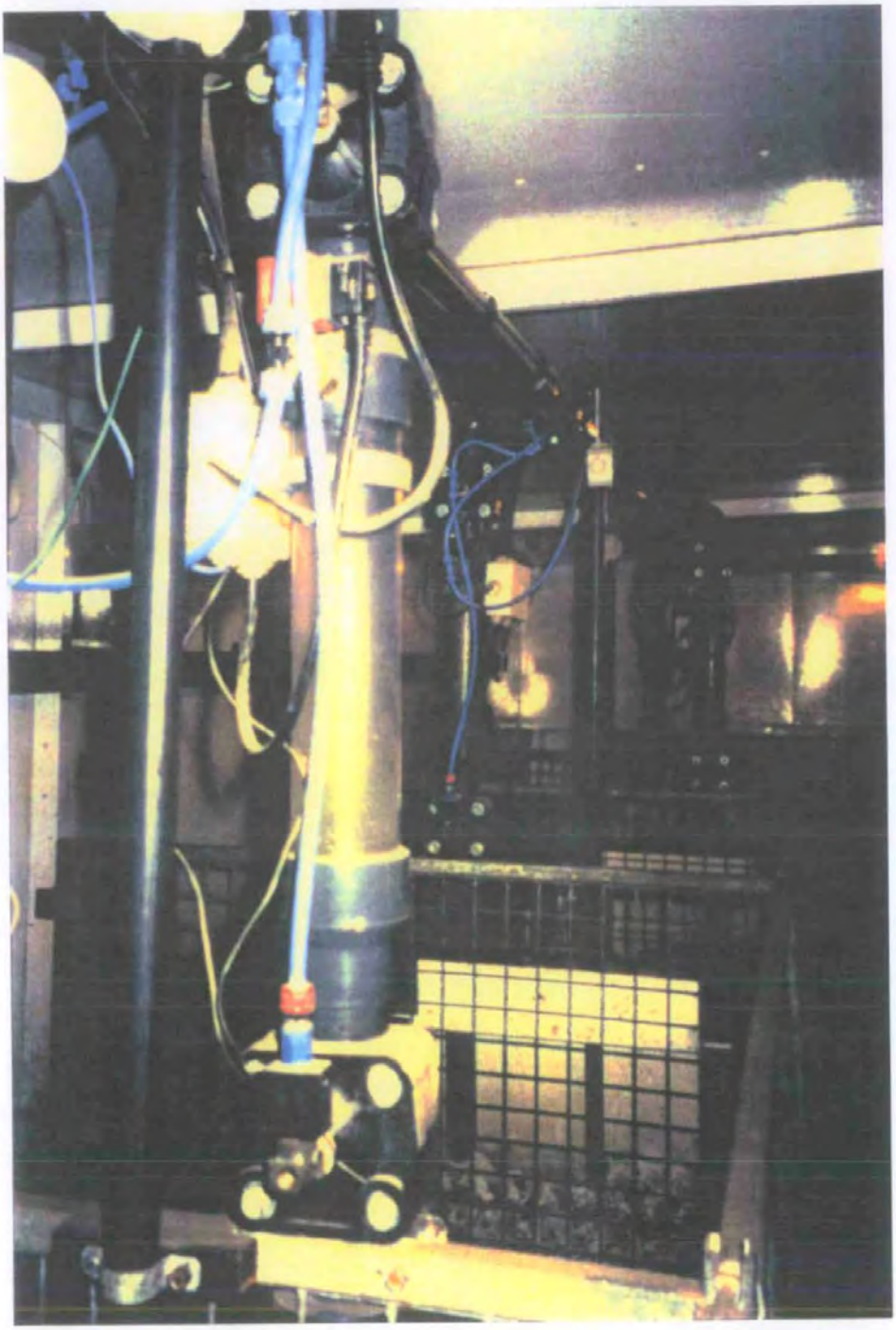
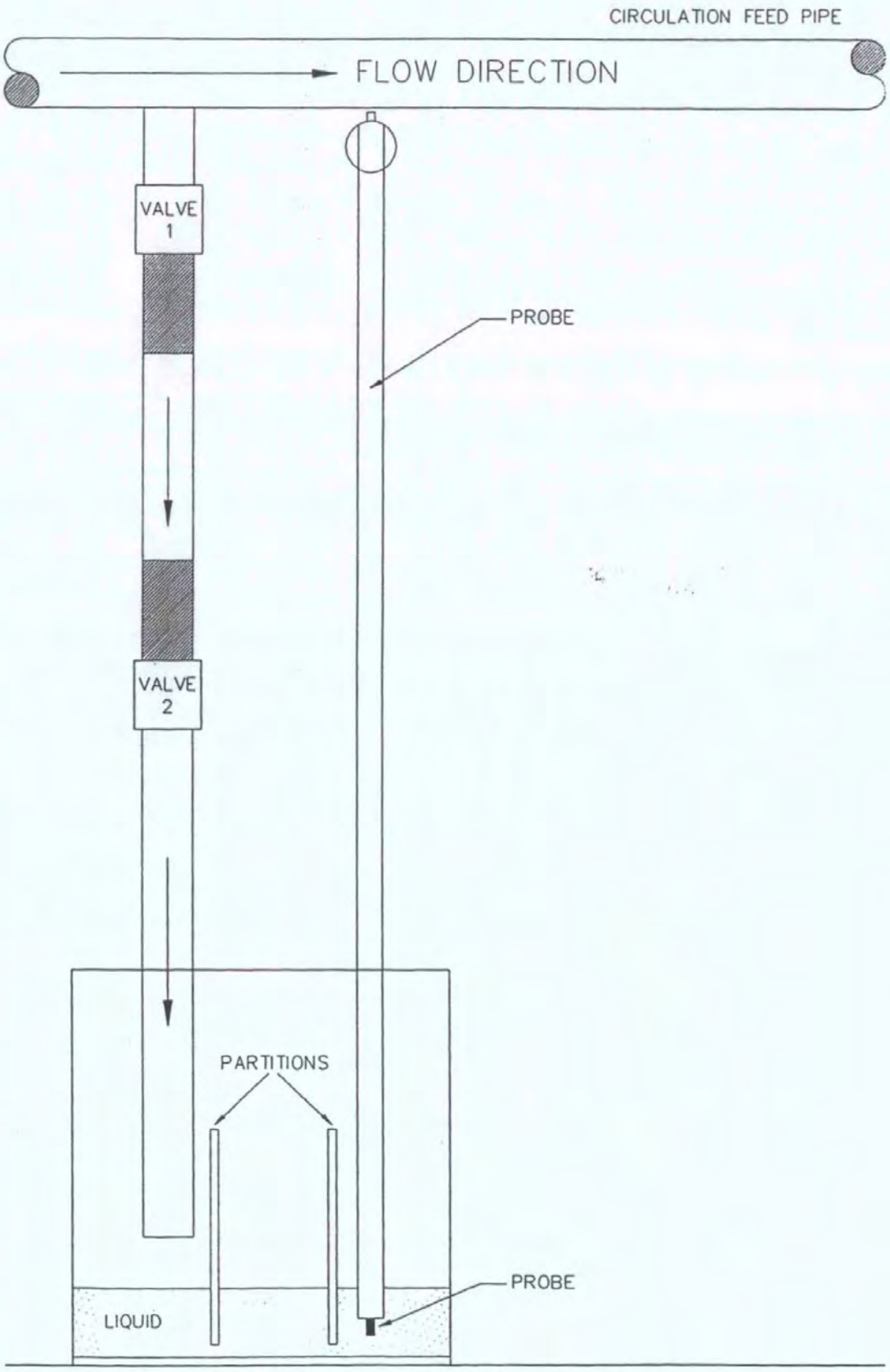


Figure 2.3 Schematic diagram of the liquid feed dispenser, feeding trough and probe.



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2.2 Analytical procedures

2.2.1 Microbiological assessments

Where microbial assessments were carried out the following procedures were adopted. Each morning prior to replenishment of the feed system, 100 ml samples of the feed mix were withdrawn after two minutes of mixing, from the liquid feed tank *via* the outfall pipe using asceptic procedures. Each sample was serially diluted in 0.25 strength Ringers solution (Unipath Ltd. Basingstoke, Hampshire) and appropriate dilutions were plated and incubated as follows. Total bacteria were assessed using the pour plate method (Banwart 1989) on Plate Count Agar (PCA) (Unipath Ltd.) incubated aerobically at 30°C for three days. Coliforms were assessed using Violet Red Bile Agar (VRBA) (Unipath Ltd.,) and the double layer pour plate method (Bridson 1990) incubated aerobically at 37°C for 24 hours in Experiment 1. In all subsequent experiments Coliforms were assessed on MacConkey's agar (MCC) (Unipath Ltd.,) using the surface plate count method (Banwart 1989) and incubated aerobically at 37°C for 24 hours. Lactic acid bacteria were assessed using the pour plate method (Banwart 1989) on de Mann, Rogosa and Sharpe, Agar (MRS) (Unipath Ltd.,), incubated anaerobically, using the gas pak system (Unipath Ltd.,) at 37°C for three days. Yeasts and mould were assessed using the plate count surface method (Banwart 1989) on Rose Bengal Chloramphenicol Agar (RBCA) (Unipath Ltd.,), incubated aerobically, at 22°C for five days. In addition the pH of each sample was measured using a pH meter (Kent EIL 7015).

2.2.2 Measurement of Alcohol (ethanol) in liquid feeds

The alcohol (ethanol) content of the liquid feed samples was determined by the distillation method for Wines (James 1995). The same volumetric flasks and density bottles were used throughout the experiment to minimise errors in volumetric variations in the glassware. The specific gravity (SG) of the samples was obtained by using Equation (2.1).

$$\text{Specific gravity} = \frac{x_2 - x_1}{x_3 - x_1} \qquad \text{(Equation 2.1)}$$

Where x_1 = weight of SG bottle empty, x_2 = weight of SG of bottle plus sample, x_3 = weight of SG bottle plus water. The relationship between the specific gravity and the proportions of alcohol (ethanol) in the liquid feed samples are presented in Appendices Table A 3.

2.2.3 Proximate Analysis of Liquid Feed Samples

Where proximate analysis of feed samples was conducted, the methods used were as described by (James 1995). In Experiment 4 and 7, proximate analysis of feed samples was undertaken by a commercial laboratory (Frank Wright, Ashbourne, Derbyshire). In this case samples were frozen within one hour of collection and stored until the end of the experiment, when the whole batch was delivered still in a frozen state to the laboratory.

2.2.4 Assessment of sugars present in the liquid feed samples

Concentrations of sugars were determined by high performance liquid chromatography (HPLC) using a Waters, Liquid Chromatograph with a refractometer detector linked to an integrator (Hewlett Packard Integrator HP3395). The eluent used was Acetonitrile (Hipersolv ^{TM} HPLC grade, BDH Chemicals Ltd) : Water 70:30 with a pump flow rate of 1.5 ml min^{-1}. Standards were prepared by diluting 1 g of each of glucose, sucrose, maltose and lactose (Analar grade, BDH Chemicals Ltd, Poole, Dorset) in a volumetric flask to 100 ml with distilled water. Standards were stored in a refrigerator at 4°C between use and renewed every three days. All liquid feed samples were prepared by stirring and sub sampling 20 ml from a 100 ml sample and centrifuged at 15,000 revolutions for 15 minutes. The resulting supernatant was withdrawn using a Pasteur pipette and forced through a 0.5 µl filter using a syringe (Whatman Intern Ltd, Maidstone, UK). Twenty µl

of the filtrate was then injected into the chromatograph. Each sample was duplicated, the peak heights obtained were recorded and the concentrations of sugars calculated against the prepared standards.

2.2.5 Measurement of Temperature in the liquid feed tanks

The temperature of the liquid diets in the liquid feed tanks was recorded using a Tiny-Talk programmable miniature temperature data logger, supplied by Orion Components Ltd., (Chichester, U.K.).

2.2.6 Pen Allocation

Siblings were allocated to consecutive pen groups in order to remove any genetic influences accoring to table A4 (Appendices).

2.3 Statistical analysis

In order to enable comparisons to be made between treatments it was necessary to use a common unit for describing the feed intake of the pig. In order to make the data consistent with normal farm recording practice dry matter daily feed intake (**DMFI**) was expressed as the quantity of fresh weight of the dry food. (Dry matter feed conversion ratio (**DMFCR**) was the appropriate multiple of **DMFI** divided by the weight gain of the pigs). Performance data were subjected to two way analysis of variance and/or analysis of covariance (using weaning age as the covariate). Data for growth rate were also analyzed using multiple regression analysis. The factors included in the analysis were weaning age and weight, sex and dietary treatments. Performance data for experiments 1, 2 and 4 were pooled and subjected to multiple and linear regression analysis. The statistical analyses were undertaken using Minitab *v* 9.2. (Minitab Inc., USA 1993). Significant differences were established using Tukey's Test (Zar 1984).

CHAPTER 3 FEEDING TRIALS

EXPERIMENT 1 PERFORMANCE, WATER CONSUMPTION AND EFFLUENT OUTPUT OF WEANER PIGS FED *AD LIBITUM* WITH EITHER DRY PELLETS OR LIQUID FEED.

3.1 Introduction

Piglets that have been suckling sows have often consumed very little solid food and drunk little water prior to weaning (Gill, *et al.* 1991). Consequently, they are unfamiliar with the idea of satisfying their hunger through the consumption of dry food. Previous studies (Brooks, Russell and Carpenter 1984; Gill, Brooks and Carpenter 1986; Gill 1989; Barber 1992) have shown that piglets will often take very large quantities of water in preference to solid feed in the first few days post weaning. When they do eventually start to eat solid feed they tend to gorge themselves. The sudden loading of the gut with solid feed can reduce the acidity of the stomach and this in turn may allow *Escherichia coli* to proliferate, precipitating diarrhoea (Schulman 1973; Sissons 1993). Water acquisition by the newly weaned pig may also be inadequate. Barber (1992) observed that while some pigs in a group may find a drinker within a few minutes of entering a pen, other pigs in the same group may take more than 24 hours, by which time they are very weak and dehydrated. Low water delivery rates from drinkers limit water consumption and this in turn limits food intake by the young pig (Barber, Brooks and Carpenter 1989).

Forbes and Walker (1968) suggested that, compared with dry feeding systems, liquid feeding systems reduce food wastage from dust. Reducing the irritant effect of high dust levels may in turn reduce pulmonary disease (Kneale 1971). Gill *et al.* (1987) suggested an additional reason for the improved performance of pigs fed liquid diets; namely that the efficiency of feed utilization is improved by presenting food in the liquid form. Barber,

Brooks and Carpenter (1991) demonstrated significant linear improvements in both dry matter and energy digestibility as the water to feed ratio increased from 2:1 to 4:1. Smith (1976) demonstrated that when feed was soaked prior to feeding *Lactobacillus* spp. proliferated, reduced the pH and as a consequence reduced the numbers of *Escherichia coli* present in the diet and that soaking the diet also improved the growth performance of the pigs.

Feeding newly weaned pigs on liquid diets is not a new practice. Indeed it was advocated by Henderson as long ago as 1814 (Henderson 1814). The feed intake of piglets, abruptly deprived of a liquid milk diet provided by their dam pre-weaning, and offered a pelleted dry diet post weaning, is often limited. It would seem logical that a piglet would adapt more readily to a liquid diet following weaning. However, the practical problems involved in providing the piglet with a continuous, wholesome, supply of liquid feed post weaning are considerable and have deterred most producers from following this course of action. Innovations in automated feed delivery systems have facilitated the *ad libitum*, liquid feeding of weaner pigs. Therefore, a reassessment of liquid feeding for newly weaned piglets is essential.

The experiment reported here was conducted to investigate whether:

- the growth performance and feed efficiency of newly weaned piglets was affected by presenting the diet as a liquid gruel as opposed to dry pellets
- water usage and effluent output of the pigs differed on the two feeding systems

3.2 Materials and Methods

3.2.1 Experimental design and treatments

Forty-eight (Trial 1) and 96 (Trial 2) Large White x (Large White x Landrace) weaner pigs (Camborough hybrids, Pig Improvement Company, Fyfield, Wick), were allocated according to a randomized block design in two trials to compare the effect of feeding newly weaned piglets *ad libitum*, on either a dry pelleted or a liquid diet. The two trials differed only in respect of the design of trough used for the liquid fed pigs. The two dietary treatments were:

DF Piglets were fed *ad libitum* on commercial, pelleted, early weaner diets. The pigs received Diet 1 for the first seven days post weaning. The diet was changed to Diet 2 over a three day period and pigs received this until the completion of the trial 28 days after weaning.

LF Piglets were fed the same diets as the **DF** pigs. The diets were provided by the manufacturer in meal form and were mixed with water in a ratio of 2.5 parts of water to one part of feed, by weight. Feed was distributed and dispensed to the pigs automatically using an *ad libitum*, liquid feed delivery system, as described in Chapter 2, 2.1.1.

3.2.2 Diets

The diets used were commercial diets manufactured by SCA Nutrition Ltd. (Thirsk, North Yorkshire). The declared nutrient composition of the diets is given in Table 3.1.

Table 3.1 Declared nutrient composition of the diets used in Experiment 1

	Diet 1	Diet 2
Digestible energy (MJ DE kg^{-1})	16.5	15.5
Crude protein (g kg^{-1})	240	230
Crude fibre (g kg^{-1})	20	25
Total ash (g kg^{-1})	60	60
Oil (g kg^{-1})	90	70
Lysine (g kg^{-1})	17	15
Vitamin A (iu kg^{-1})	16,000	16,000
Vitamin D3 (iu kg^{-1})	2,000	2,000
Vitamin E (iu kg^{-1})	250	250
Selenium (mg kg^{-1})	0.3	0.3
Avilamycin [a] (mg kg^{-1})	40	40
Copper sulphate (mg kg^{-1})	175	175

[a] Avilamycin (Maxus, Elanco Products Ltd, Basingstoke, Hampshire)

Diet 1 contained approximately 50% cooked cereals in the form of porridge oats, cooked maize and cooked wheat, in a ratio of approximately 5:4:1. Milk products (primarily skim milk powder with a small inclusion of whey powder) contributed 15 - 16% lactose in the final diet. In addition a small quantity of sucrose was added. Additional protein was supplied as steam dried fish meal (68 - 70% protein; 10 - 12% oil) and additional fat as soya bean oil. In Diet 2 the cereal component (approximately 50%) comprised cooked wheat, cooked maize and porridge oats in a ratio of approximately 6:3:1. The main milk product present was whey powder with a small quantity of skim milk powder. Together these contributed 8 - 9 % lactose in the final diet. Additional protein was supplied as steam dried fish meal (68 - 70% protein; 10 - 12% oil) and a small quantity of Hipro soya (solvent extracted soya bean meal, 49% protein; 1 - 2 % oil, North American). Trace quantities of sucrose, starch and wheatfeed were included. Both diets were supplemented with minerals, trace elements and vitamins.

3.2.3 Trial procedure

Pigs of similar weight, weaned at a mean age of 22.6 ± 2.6 days, were randomly allocated to pen groups comprising six piglets. Treatments were replicated four times. A replicate consisted of four pen groups (24 pigs); two pen groups of pigs were randomly allocated to the **DF** and two to the **LF** treatment. Within a replicate the number of males and females per pen group was kept constant. Trials 1 and 2 had two (total 48 pigs) and four (total 96 pigs) replicates respectively. The pigs were maintained on their respective treatments for 28 days and were weighed at weekly intervals throughout the trials. The health of the animals was monitored closely and all veterinary interventions were recorded.

Feed and water were introduced to the mixing/recirculation tank at the commencement of the trial. The operation of the system resulted in a quantity of the feed/water mixture remaining in the mixing tank at the end of each 24 hour period. In Trial 1 the tank was replenished as necessary and usually on a daily basis. Meal and water, in the correct proportions, were added to the residual feed in the tank. In Trial 2 more attention was paid to standardising the amount of feed being carried forward to the following day. The aim was to use approximately half of the feed in the tank each day leaving the residue to act as an inoculum for the new feed which was added.

In Trial 2 a microbiological assessment was made of the liquid feed mixture during the time that two different replicates (Replicate 1 and Replicate 4) of animals were on trial. Each morning prior to replenishment, samples of the liquid feed mixture were removed from the mixing tank using aseptic procedures. Samples collected during the time that Replicate 1 was on trial were plated within two hours of collection. Samples collected during the time that Replicate 4 was on trial samples were stored in a refrigerator at 4°C for up to 48 hours before the microbial assessment was conducted.

3.3 Results

3.3.1 Animal health

There were no major problems with the health of the animals. Some mild scouring occurred in Trial 1 during a period of very hot weather. Affected animals were treated with Apralan (Elanco Products Ltd, Basingstoke) in their drinking water for three days. A total of eleven pigs (eight **DF** and three **LF**) were treated for joint-ill with one ml injections of Duphamox (Solvay Duphar Veterinary, Southampton) on three consecutive days. In addition three pigs (two **DF** and one **LF**), whose performance was being compromised by infection with joint-ill were removed from the trial. One pig was removed from the liquid feeding-treatment (Trial 2) because it refused to eat. This pig was subsequently offered dry pelleted feed which it consumed readily and subsequently exhibited a normal growth rate. Pigs on **LF** sometimes had rather loose faeces but without evidence of scour other than in the cases noted above.

3.3.2 Growth and feed utilisation

The biological performance of the pigs in Trials 1 and 2 are summarized in tables 3.2 and 3.3. Data for daily gain in each week of the trial and for the entire four week trial period were subjected to multiple regression analysis. All the analyses were highly significant ($P<0.001$). In the first week following weaning the age of the pig at weaning accounted for 0.79 ($P<0.001$) and dietary treatment only 0.14 ($P=0.084$) of the variation in growth rate. Over the total four week period dietary treatment accounted for 0.52 ($P<0.001$) and weaning age 0.37 ($P=0.002$) of the variation in growth rate. Neither sex nor weaning weight were significant contributors to the variation in growth rate. Therefore, the data were subjected to analysis of covariance using weaning age as the covariate.

Table 3.2 Performance of pigs fed dry pelleted or liquid diets (Trial 1)

Parameter	Period	Dry pelleted feed	Liquid feed	s.e.d
Dry matter feed intake (g d^{-1})	week 1	130	416	11 ***
	week 2	354	741	19 ***
	week 3	636	1068	16 ***
	week 4	889	1204	35
	Overall	**443**	**807**	**11 *****
Daily gain (g d^{-1})	week 1	62	123	23 ***
	week 2	264	426	37 ***
	week 3	529	635	38 ***
	week 4	674	630	44
	Overall	**343**	**428**	**21 *****
Dry matter Feed conversion ratio	week 1	3.36	3.47	0.67
	week 2	1.36	1.75	0.05 *
	week 3	1.24	1.69	0.03 **
	week 4	2.29	1.91	0.38
	Overall	**1.31**	**1.89**	**0.03 ** **
Total water intake (ml pig d^{-1})	week 1	689	1341	38 **
	week 2	993	2022	77 **
	week 3	1910	2955	88 **
	week 4	2065	3428	93 **
	Overall	**1306**	**2298**	**64 ** **
Average effluent production (ml pig d^{-1})	week 1	337	510	13 *
	week 2	439	730	46
	week 3	1144	1510	137
	week 4	965	1852	131
	Overall	**754**	**1058**	**46 ***

* $P<0.05$; ** $P<0.01$; *** $P<0.001$.

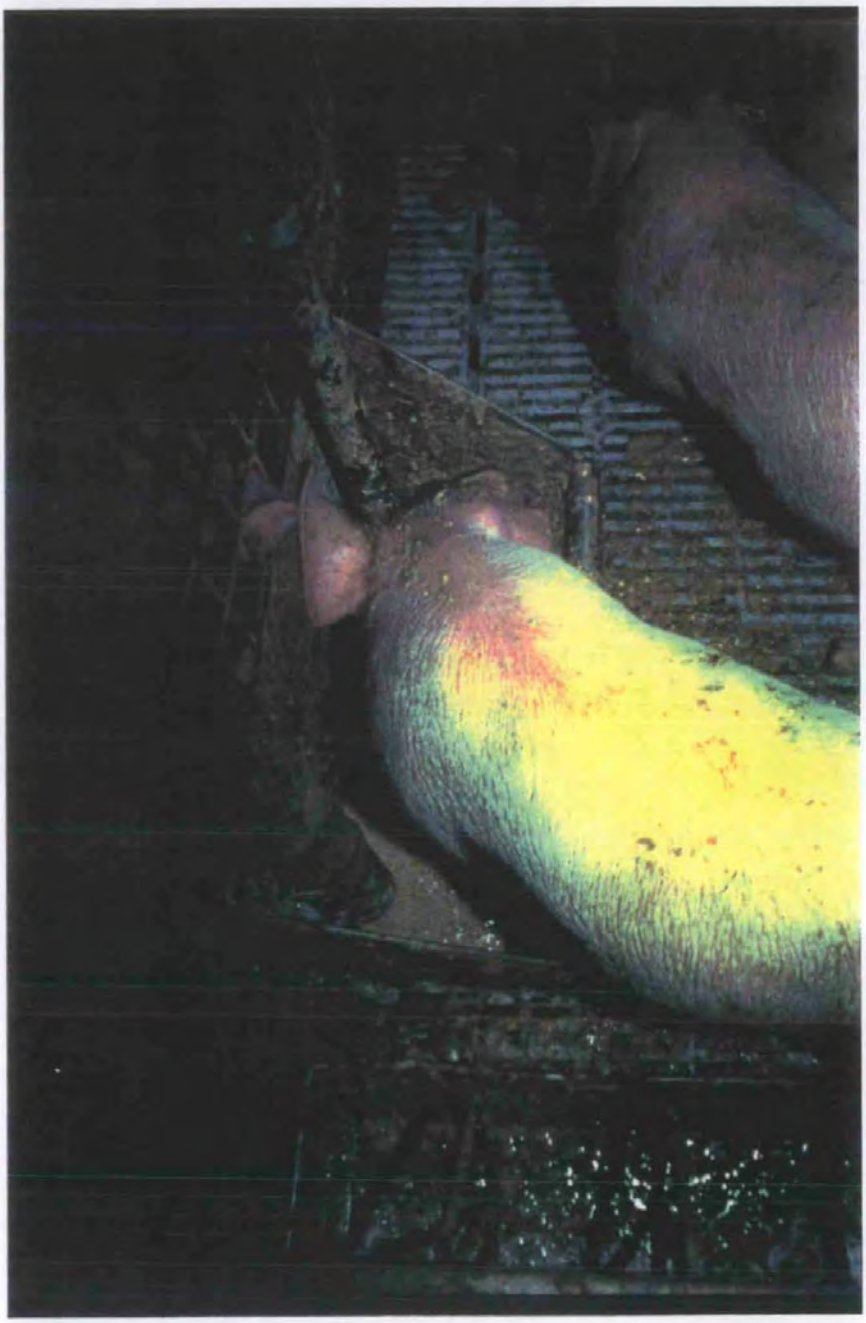
Table 3.3 Performance of pigs fed dry pelleted or liquid diets (Trial 2)

Parameter	Period	Dry pelleted feed	Liquid feed	s.e.d.
Dry matter feed intake (g d^{-1})	week 1	199	271	9 *
	week 2	418	560	16 ***
	week 3	686	819	12 ***
	week 4	877	964	9 **
	Overall	**545**	**654**	**10** ***
Daily gain (g d^{-1})	week 1	140	178	21
	week 2	340	425	28 **
	week 3	511	602	21 ***
	week 4	594	610	22
	Overall	**397**	**454**	**14** ***
Dry matter feed conversion ratio	week 1	1.52	1.57	0.08
	week 2	1.25	1.36	0.02
	week 3	1.34	1.37	0.02
	week 4	1.48	1.58	0.01 *
	Overall	**1.37**	**1.44**	**0.01** **
Total water intake (ml pig d^{-1})	week 1	641	958	36 ***
	week 2	1173	1698	49 ***
	week 3	1827	2473	68 ***
	week 4	2355	2984	92 *
	Overall	**1499**	**2028**	**84** **
Average effluent production (ml pig d^{-1})	week 1	409	575	50
	week 2	722	883	57
	week 3	1200	1556	88
	week 4	1570	1844	64
	Overall	**982**	**1189**	**31** *

* $P<0.05$; ** $P<0.01$; *** $P<0.001$.

In trial 1 the DMFI was significantly ($P<0.001$) greater for the **LF** pigs. Overall feed intake appeared to be 82% greater. The DMFI of **LF** pigs was greater in Trial 1 than Trial 2 (416 *v* 271 g d^{-1}). However, this is misleading, because of the high level of feed wastage in Trial 1. Although the DMFI figure overestimates consumption the growth rate of **LF** pigs was significantly greater that **DF** pigs in weeks 1, 2, 3 and for the overall 28 day period post weaning. On average, **LF** pigs weighed 2.4 kg more at 28 days post weaning than **DF** pigs. Dry matter feed conversion ration (DMFCR), was significantly ($P<0.01$) inferior in the **LF** pigs (1.89 *v* 1.31 or 44%). Behavioral observations of the pigs indicated that much of this difference could be attributed to increased wastage by the pigs on the **LF** treatment. The design of the troughs resulted in considerable wastage and the pigs spilled copious quantities of the wet feed as they jostled at the trough (Plate 3.1). In addition the pigs carried considerable amounts of feed away from the trough due to it sticking to their heads and ears. The wastage losses were unacceptably high and resulted in both the **LF** pigs and their pens looking dirty. Consequently, the trough design was changed in Trial 2 in an attempt to reduce losses due to feed wastage (Plate 3.2).

In Trial 2 the DMFI of the **LF** pigs was significantly ($P<0.05$ to $P<0.001$) greater in each of the four weeks post weaning and overall ($P<0.001$) for the **LF** pigs. In this case the **LF** pigs apparently consumed 20% more feed than the **DF** pigs in the 28 days post weaning. Feed wastage was notably reduced and as a consequence DMFCR was not significantly different between the **LF** and **DF** pigs in weeks 1 - 3 post weaning. However, DMFCR was approximately 7% poorer in week four ($P<0.05$) and 5% poorer overall ($P<0.01$). Daily gain of the **LF** pigs was significantly ($P<0.001$) greater than that of the **DF** pigs in weeks 2 and 3 post weaning and overall. However, the differences were not statistically significant in weeks 1 and 4. **LF** pigs gained 57±14 g d^{-1} faster that the **DF** pigs overall and as a consequence were on average 1.6 kg heavier at 28 days post weaning.



3.3.3 Water usage and effluent production

Water usage was monitored in each of the trials. The values for total water usage presented in tables 3.2 and 3.3 represent the quantity of water taken from the drinkers in the case of the **DF** pigs and the sum of water from the drinkers and water mixed with feed in the case of the **LF** pigs. The water usage of **LF** pigs was significantly greater than that of **DF** pigs in each week, and overall, in both trials. In Trial 1 **LF** pigs took on average 76% more water in Trial 2 35% more water than **DF** pigs. In both trials differences in the weekly values for effluent output were not statistically significant but the differences in overall output of effluent were ($P<0.05$). In Trial 1 the effluent output of **LF** pigs was 40% greater than **DF** pigs and in Trial 2 output was 21% greater.

3.3.4 Microbiology of the liquid feed system

The microbiology of the liquid feed was studied in two replicates (Replicates 1 and 4). The changes in the $\log_{10}$ numbers of coliforms, lactic acid bacteria and total organisms, with time, is shown in figure 3.1 a,b. The number of organisms in the feed increased rapidly over the first five days and thereafter showed a high degree of stability. In Replicate 1 both total organisms and total lactic acid bacteria stabilized at a higher level than in Replicate 4. The total coliforms found in the diet differed between the two replicates. In Replicate 1 coliforms were present in appreciable numbers for only the first few days of the trial and intermittently, in small numbers, thereafter. In Replicate 4 coliforms were present in higher numbers and more consistently during the 28 day period. The changes in lactic acid bacteria numbers and pH are shown in figures 3.2 a,b. and the relationship between coliform numbers and the pH of the feed are shown in figure 3.3 a,b. Increased numbers of lactic acid bacteria in Replicate 1 resulted in a lower pH *circa* 3.5. Coliform numbers were generally low or undetectable from the ninth day of the trial.

Figure 3.1 Changes in the number of total organisms, coliforms and lactic acid bacteria in the liquid feed system with time (Trial 2).

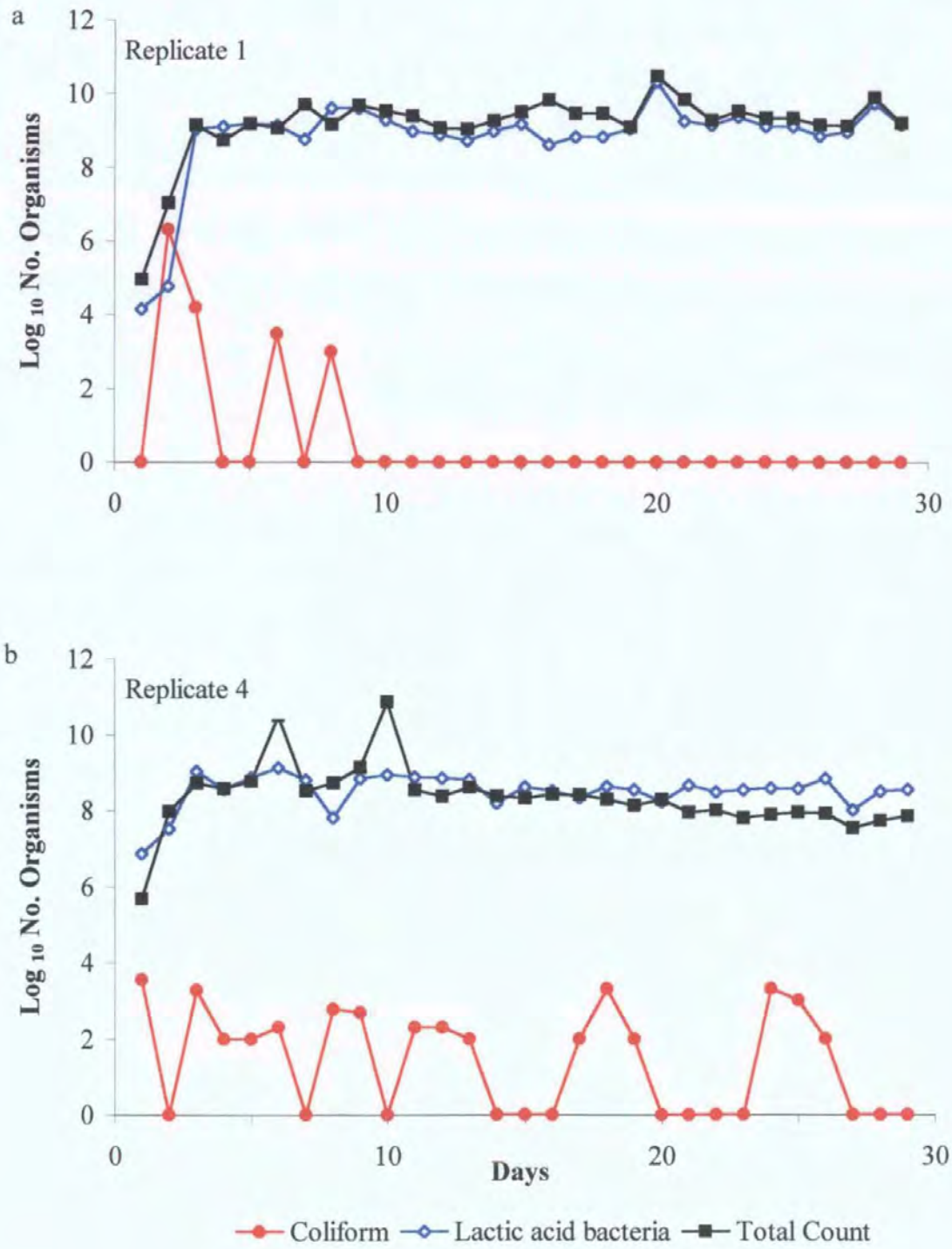


Figure 3.2 Relationship between lactic acid bacteria and pH in the liquid feeding system with time.

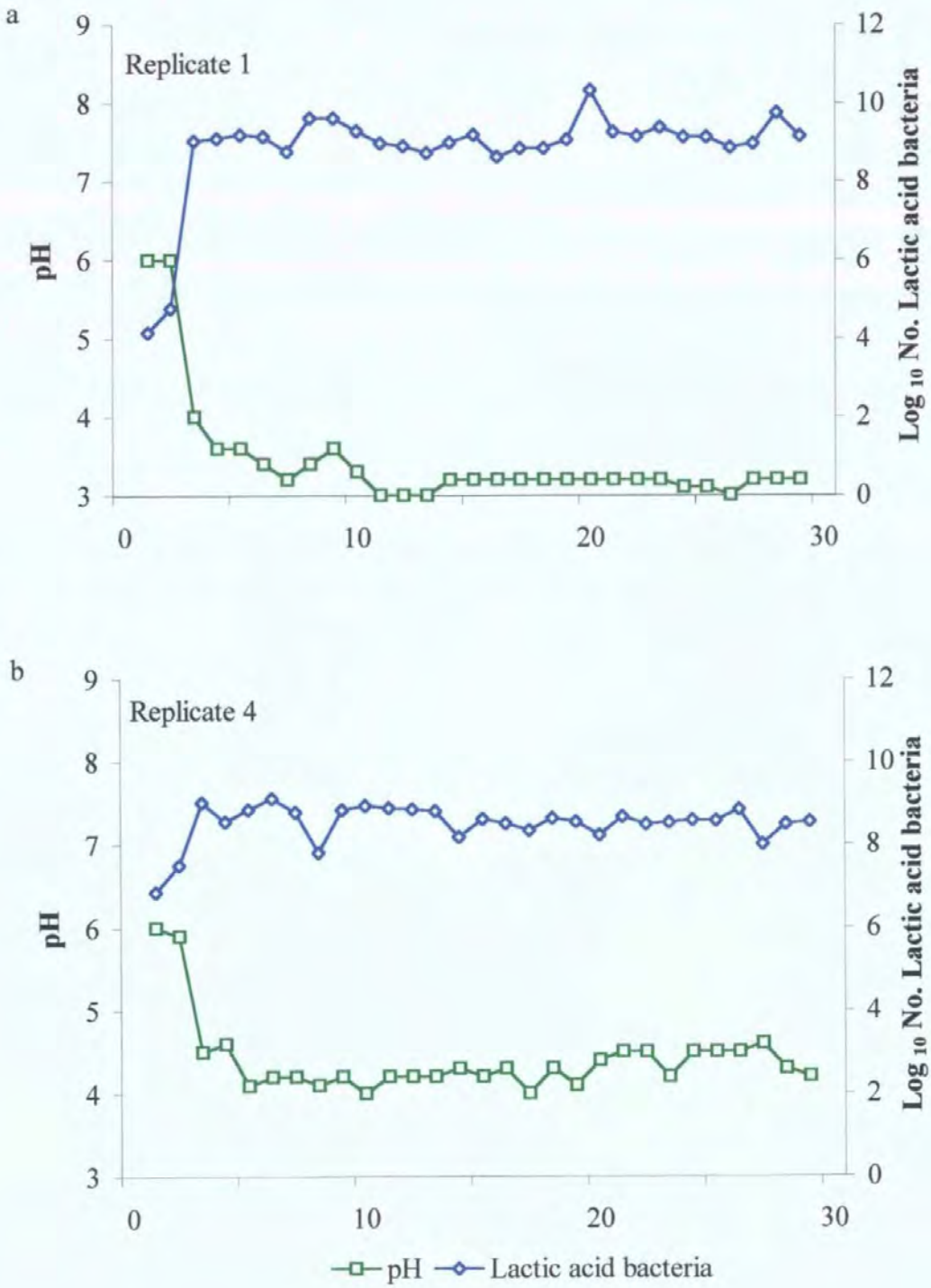
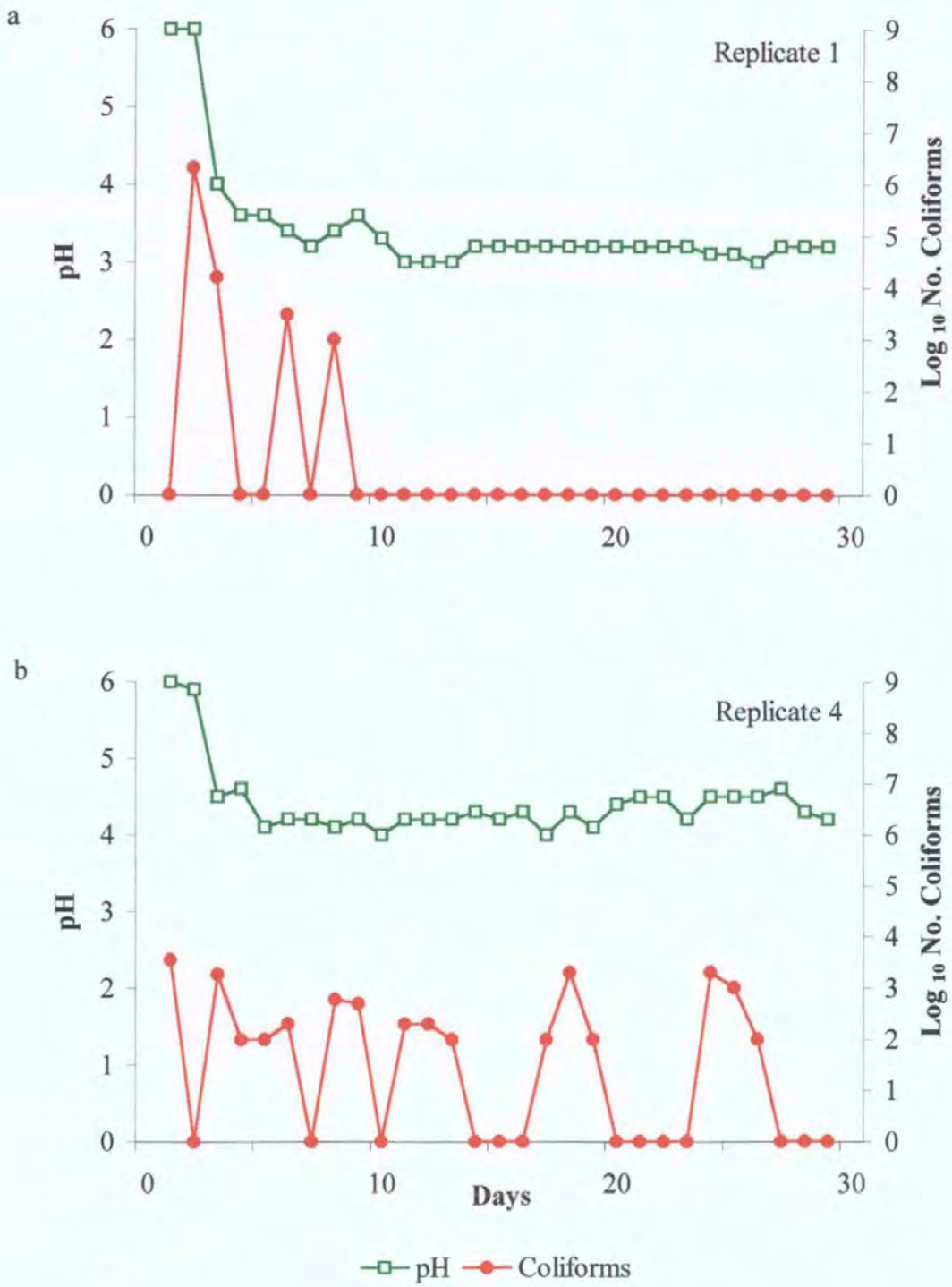


Figure 3.3 Relationship between pH and coliform numbers in the liquid feeding system with time.



In Replicate 4 the lactic acid bacteria numbers were lower and the pH higher (circa 4.0) than in Replicate 1. Coliforms were found in the liquid feed mixture, intermittently, throughout the trial.

3.4 Discussion and Conclusions

Because the intake of solid food by newly weaned pigs is low both commercial pig producers and nutritionists assume that the appetite of the newly weaned pig is small and a limiting factor to intake (Bark, *et al.* 1986; Toplis 1992). However, piglets have been dealing with a dilute feed source while on the sow and their gut capacity is increasing very rapidly at the time of weaning Kvasnitskii cited by (Kidder and Manners 1978). The results obtained in this experiment demonstrate that the dry matter feed intake of the newly weaned pig can be increased by providing it with a liquid diet even though that implies the pig consuming a greater volume of material. One reason for the increased consumption of dry matter by pigs fed a liquid diet is that the weaners do not have to learn separate patterns of feeding and drinking behaviour, most of their requirements for both food and water are met at a single delivery point (Partridge and Gill 1993). On a solid diet the piglet may go for a long period post weaning without eating and then gorge itself with food when is 'discovers' the feed trough (Sissons 1993) whereas a liquid diet may produce a more regular pattern of feeding more quickly after weaning. Another advantage of liquid diets is that they help ensure that the piglet takes liquid and hence prevent it from becoming dehydrated. In the two trials reported here, piglets offered liquid feed had higher feed intakes in the first week post weaning than piglets offered dry feed. The difference in intake in the first week post weaning was statistically significant in both trials.

During both of the trials reported here, **LF** pigs grew 25 and 14% faster than **DF** pigs during the four weeks post weaning. An improvement in growth associated with the

feeding of liquid diets is consistent with the findings of other authors (Braude and Newport 1977; English, Anderson, Davies and Dias 1981; Taverner, Reale and Campbell 1987; Lecce, *et al.* 1979; Partridge, Fisher, Gregory and Prior 1992) and in contrast with the study of Kornegay and Thomas (1981) who found no significant improvement in performance as a result of feeding pigs liquid diets. The improvement in daily gain on liquid diets may be a result of increased intake of feed and increased water intake. Previous work by Barber *et al.* (1989) has shown that increasing the water consumption of young pigs results in improved growth and, subsequent work by (Gill *et al.* 1987) and (Barber *et al.* 1991) showed that providing feed mixed with water improves the utilisation of dry matter. In the trials reported here FCR was better on dry diets than on liquid ones. However, this may be a reflection of differences in feed wastage on the two diets rather than of differences in efficiency of feed utilization. Unfortunately, it was not possible to compare the two trough designs in a single study therefore it is not possible to assume that the differences were entirely due to that factor. Nevertheless, visual observation of the pigs and the pens suggested that the improved trough design reduced feed wastage and this would certainly have contributed to the improvement in FCR. If the actual utilization of feed by **LF** pigs was similar in both trials then the improvement in trough design would have been representative of a 24% reduction in feed wastage. Despite the improvement in the FCR in Trial 2 the **LF** pigs still had a significantly poorer FCR ($P<0.01$) than that of the **DF** pigs. This suggests that the design of troughs for liquid feeding young pigs may still require further improvement to reduce wastage, as many other studies have shown improved FCR when pigs are fed liquid diets (Braude 1972; Braude 1990).

In view of the increased feed and water intake by the **LF** pigs it is hardly surprising that the average daily effluent output was greater on the liquid feeding system. In Trial 2, using the improved trough design, the difference in effluent output between **DF** and **LF** pigs was

21% compared with 40% in Trial 1. However, even this figure is somewhat misleading because the **LF** pigs were not only eating and drinking more but also growing faster. If the effluent output per unit gain is calculated for **DF** and **LF** pigs in both trials it is found to be 2.20 and 2.47 in Trial 1 (a difference of 12%) and 2.47 and 2.61 in Trial 2 (a difference of 5.7%). Thus the additional effluent produced using the better **LF** system was 6% and not 21%.

It became obvious in the course of the first trial that the liquid feed system acted as a fermentation vessel. The diets provided a good source of lactose and other readily fermentable carbohydrate and became colonized by lactic acid bacteria. Because the system was never allowed to empty completely once fermentation had started, an inoculum was carried over in the feed from one day to the next. In the second trial a deliberate attempt was made to standardize this process by carrying forward approximately half the feed each day to encourage fermentation. A microbiological analysis of the diet was undertaken for two of the replicates. The mixing tanks were open to the atmosphere. Therefore, the microbial flora which developed in the feed system originated either from the dry diet used or from atmospheric inoculation. The whole system was cleaned out and sterilized between replicates, so for each new replicate the feed system had to develop its microflora anew. It is interesting to note the difference between the two feeding periods tested. In Replicate 1, lactic acid bacteria growth resulted in a pH around 3.5. This pH appeared to inhibit coliform growth. Coliforms were only occasionally detected after the first week, usually immediately after the tank had been replenished, when the pH of the feed mix was temporarily increased. In Replicate 4, lactic acid bacteria numbers never reached the levels recorded in Replicate 1. As a consequence the pH of the feed mix never dropped below 4.0. At this pH coliforms were still able to survive and for most of the feeding period the feed contained some coliforms. The reasons for the difference in Lactic acid bacteria

growth and pH between the two replicates is not readily apparent. Three possible explanations may be offered. Firstly, a feed delivery took place between the two series of tests. Therefore, the differences may have resulted from small variations in the formulation of the feed effectively altering the substrate available to the lactic acid bacteria. A second possibility is that small difference in raw materials may have increased the buffering capacity of the diet used in Replicate 4 and thereby have prevented the pH from falling to such a low level as in Replicate 1. The third possibility is that the feed system was colonized by a different strain or strains of lactic acid bacteria with a higher pH threshold. It is clear that in some circumstances a very favourable fermentation pattern can occur which will lower the pH to the point where the diet is devoid of coliforms. Such circumstances should benefit the pig as it will then be less challenged by coliforms of dietary origin. Furthermore, the low pH of the diet will assist in maintaining a low pH in the stomach and thereby assist in preventing the development of coliform scours (Easter, 1993).

In summary, the results from this experiment indicate that liquid feeding piglets in their first few weeks after weaning resulted in increased post weaning growth rates. If fermentation patterns of liquid feed can be successfully controlled, liquid feeding may also assist in the prevention of coliform scours and consequently the problems of malabsorption that they often produce. If both these features can be achieved at the same time, with predictability, then the benefits in terms of lifetime growth and feed efficiency will be considerable.

EXPERIMENT 2 PERFORMANCE OF WEANER PIGS FED *AD LIBITUM* WITH LIQUID FEED AT FOUR DIFFERENT DRY MATTER CONCENTRATIONS.

3.5 Introduction

In Experiment 1, it was found that liquid feed was well accepted by newly weaned piglets. Liquid feeding was also shown to have increased the dry matter intake and weight gain of piglets during the first four weeks post weaning.

Smith (1976) showed that *Lactobacillus* spp. which occur naturally on cereal grains, will proliferate in liquid feed and produce acidic conditions. The liquid feeding system used in the Experiment 1 was found to act as a fermentation vessel resulting in a rapid development of lactic acid bacteria over the first five days of operation. As a consequence of this fermentation the pH of the liquid feed fell below 4.0 and the growth of *Escherichia coli* was suppressed.

Work with older pigs has shown that the efficiency with which feed was utilised was improved by increasing the proportion of liquid to the dry matter fraction (Gill 1989). Subsequent work by Barber *et al.* (1991), demonstrated that increasing the water to feed ratio improved both dry matter and energy digestibility. Commercial producers are feeding weaner pigs diets with a variety of dry matter contents ranging from 2.1:1 to 5:1 and reporting good results (P. McTiffin, personal communication 1995). However, there is no research information on which to base a recommendation for the ideal dry matter content of liquid diets for pigs of this age.

Yang *et al.* (1981) suggested that abdominal fill may be important in the regulation of

voluntary food intake. In their study pigs of 30 kg were used and they concluded that the daily intake of dry matter and water (total volumetric intake) of the growing pig was 19% of live weight. Below this limit the pig would consume food as a first requirement and limit water to a minimum level. Thus the minimum intake of water per unit of feed dry matter would occur when the pig was fed a dry diet *ad libitum* and had access to a readily available supply of water. Barber (1992) conducted similar experiments with older pigs, weighing between 30 and 60 kg, and confirmed that volumetric intake was a limiting factor on dry matter intake, and that in these heavier pigs total volumetric intake represented only 12% of live weight. Therefore, it would be anticipated that the volume of liquid feed that pigs need to consume to satisfy their nutrient requirements may in itself limit dry matter intake, especially where very dilute liquid feeds are used.

The objectives of this experiment were:

- to compare the effect on intake and performance of a range of dietary dry matter concentrations representative of those being used in commercial practice on newly weaned piglets
- to assess whether the increasing dilution of feed affected the pattern of microbial activity in the system and the output of effluent

3.6 Materials and Methods

3.6.1 Experimental facilities

These have been described previously in Chapter 2, 2.1.

3.6.2 Experimental design and treatments

Ninety six weaner pigs, Large white x (Large white x Landrace) Camborough hybrids, (Pig

Improvement Company, Fyfield, Wick), were allocated according to a randomized block design to four dietary treatments which differed only in dry matter concentration. Liquid diets with dry matter concentrations of 149, 179, 224 and 255 g kg^{-1} were prepared by mixing commercial, pelleted, early weaner diets in the ratios of 5:1, 4:1, 3:1 and 2.5:1 water to feed, (treatments **DM149, DM179, DM224, DM255** respectively).

3.6.3 Diet preparation and feeding

The diets were prepared by mixing commercial, pelleted, early weaner diets with water by weight to give a dry matter concentration of 149, 179, 224 and 255 g kg^{-1}. The pigs received Diet 1 for the first seven days post weaning. Diet 2 was fed for the remainder of the trial. Residual feed in the tank ensured that the change from Diet 1 to Diet 2 was a gradual process. Feed was distributed and dispensed to the pigs automatically using the *ad libitum*, liquid feed delivery system described in Chapter 2, 2.1.1.

3.6.4 Diets

The diets used were standard commercial diets manufactured by Roche Products Ltd., (Heanor, Derbyshire). The declared nutrient composition of the diets is given in table 3.4.

Diets 1 and 2 contained approximately 50% cooked cereals in the form of porridge oats, cooked maize and cooked wheat, in a ratio of approximately 2:2:4. Milk products (skim and whey powder) contributed 15% lactose to the final diet. In addition a small quantity of sucrose was added. Additional protein was supplied as steam dried fish meal (68 - 70% protein; 10 - 12% oil) and a processed soya bean product (solvent extracted soya bean meal). Additional oil was supplied by soya bean oil. In Diet 2 the cereal component (53%) comprised cooked and uncooked wheat (1:1), cooked maize and porridge oats in a ratio of approximately 6:3:1. The main milk product present was whey powder

contributing 10 - 11% lactose in the final diet. Additional protein was supplied as steam dried fish meal (68 - 70% protein; 10 - 12% oil), processed soya bean meal (solvent extracted soya bean meal) and full fat soya (whole soya beans, cooked and milled to provide 36% protein and 18% oil). A small amount of sucrose was included. Both diets were supplemented with minerals, trace elements and vitamins.

Table 3.4 Declared nutrient composition of the diets used in Experiment 2.

	Diet 1	Diet 2
Digestible energy (MJ DE kg^{-1})	16.5	15.5
Crude protein (g kg^{-1})	225	220
Crude fibre (g kg^{-1})	20	24
Total ash (g kg^{-1})	60	60
Oil (g kg^{-1})	90	75
Lysine (g kg^{-1})	15.5	15
Vitamin A (iu kg^{-1})	16,000	16,000
Vitamin D3 (iu kg^{-1})	2,000	2,000
Vitamin E (iu kg^{-1})	250	250
Selenium (mg kg^{-1})	0.3	0.3
Avilamycin [a] (mg kg^{-1})	40	40
Copper sulphate (mg kg^{-1})	175	175

[a] Avilamycin (Maxus, Elanco Products Ltd, Basingstoke, Hampshire)

3.6.5 Trial procedure

Piglets of similar weight (7.17 ± 0.82 kg) and age (24 ± 2.6 days), were randomly allocated to pen groups comprising six piglets. Treatments were replicated four times. A replicate consisted of four pen groups (24 pigs); randomly allocated to treatments **DM255, DM224, DM179,** and **DM149**. Within a replicate the number of males and females were equal. The piglets were maintained on treatment for 28 days. Piglets were weighed at weekly intervals throughout the experimental period. The health of the animals was monitored closely and all medications and veterinary interventions recorded.

Feed and water were introduced to the mixing/recirculation tank three days before each

treatment began in order to permit microbial activity to take place before feeding to the piglets. The system was operated to ensure that a quantity of the feed/water mixture remained in the mixing tank at the end of each 24 hour period. The aim was to use approximately half of the feed in the tank each day leaving the residue to act as an inoculum for the new feed which was added.

A microbiological assessment was made of the liquid feed mixture for each treatment. The feeding system was cleaned prior to the introduction of new treatments to the facility. Each morning prior to replenishment of the feed system, samples of the feed mix were removed after two minutes of mixing from the mixing tank *via* the outfall pipe using aseptic procedures. A microbiological assessment was conducted as described previously (Chapter 2, 2.2.1.

The temperature of the liquid feed tanks was monitored using a Tinytalk-Temp described previously (Chapter 2, 2.2.5), which was suspended in the middle of the mixing tank completely immersed in liquid feed. In addition to the liquid diet clean water was freely available to the pigs from nipple waterers at all times.

3.6.6 Statistical Analysis

Feed intake was calculated on a dry matter only basis (**DMFI**). Feed conversion ratio (**DMFCR**) was the appropriate multiple of **DMFI** divided by the weight gain of the pigs. Performance data were subjected to two way analysis of variance. Daily gain was also analyzed using covariance analysis (using weaning age as the covariate). All statistical analyses were undertaken using Minitab version 9.2. (Minitab Inc., State College, USA 1993). Significant treatment differences were established using Tukey's Test (Zar 1984).

3.7 Results

3.7.1 Animal health

There were some minor health problems, which are summarised in table 3.5

Table 3.5 Veterinary interventions and medications (Experiment 2)

Problem	Dry matter content of liquid diet g kg^{-1}				Intervention
	149	179	224	255	
Joint ill		4	1	2	1 ml injection of Duphamox[a] 3 consecutive days
Enzootic pneumonia		1	3		Removed from trial
Ear infection				1	1 ml injection of Duphapen[b] followed by two 1 ml injections of Duphamox
Skin rash	1				2 ml injection of Multivet[c] combined with 1 ml injection of Duphapen

[a] Duphamox (Solvay Duphar Veterinary, Southampton)
[b] Duphapen (Solvay Duphar Veterinary, Southampton)
[c] Multivet 4BC (C-Vet Ltd, Bury St Edmonds)

but none of these appeared to be treatment related. Some mild scouring occurred on **DM179** but did not require medication. One pig on **DM149** refused to eat liquid feed and was removed from the trial. This pig was offered dry pelleted feed which it consumed readily and subsequently exhibited a normal growth rate.

3.7.2 Growth and feed utilisation

The biological performance of the pigs is summarized in table 3.6.

Table 3.6 Mean post weaning performance of pigs fed fermented liquid feed with dry matter content of 255, 224, 179 or 149 g kg^{-1}.

Parameter	Period	Dry matter content of liquid diet g kg^{-1}				s.e.d.
		255	224	179	149	
Dry matter feed intake (g d^{-1})	week 1	204[a]	178[b]	109[ab]	161	19**
	week 2	397[a]	367	380	320[a]	24*
	week 3	600	539	599	578	26
	week 4	700	648	774	746	36
	Overall	475	433	466	451	22
Daily gain (g d^{-1})	week 1	187[ab]	131	74[a]	90[b]	35**
	week 2	395	322	356	354	27
	week 3	577[ac]	427[abd]	504[cd]	516[b]	36**
	week 4	449[ab]	465[c]	531[b]	543[ac]	42*
	Overall	403[a]	344[a]	366	380	22*
Dry matter feed conversion ratio	week 1	1.15[a]	1.35	1.39	1.95[a]	.24*
	week 2	1.02	1.13[a]	1.00	0.92[a]	.06*
	week 3	1.05[a]	1.25[a]	1.18	1.12	.06*
	week 4	1.60	1.38	1.40	1.39	.11
	Overall	1.20	1.22	1.23	1.20	.03
Total water intake (ml pig d^{-1})	week 1	899	858	738	1037	88
	week 2	1390[ab]	1532[c]	1878[b]	1944[ac]	131*
	week 3	2180[bd]	2139[ac]	3049[cd]	3568[ab]	183***
	week 4	2782[bd]	2629[ac]	3778[cd]	4658[ab]	331**
	Overall	1813[bd]	1790[ac]	2361[cd]	2802[ab]	167**
Average water intake from drinkers (ml pig d^{-1})	week 1	306	241	249	118	98
	week 2	227	251	145	111	76
	week 3	423	258	287	256	138
	week 4	735[ab]	368[b]	230[a]	383	137*
	Overall	423	280	228	217	101
Average effluent production (ml pig d^{-1})	week 1	512	540[bc]	69[abc]	819[ade]	94***
	week 2	679[ac]	1012[b]	1534[ab]	1153[c]	142**
	week 3	1044[a]	1245[b]	1647	2377[ab]	272**
	week 4	1904[a]	1990[b]	2533[c]	3441[abc]	303**
	Overall	1034[a]	1197[b]	1446[c]	1948[abc]	144**

[a,b,c] Means with the same superscript in the same row differ significantly (P<0.05).
* P<0.05; ** P<0.01; *** P<0.001.

Taken over the whole 28 days, treatment had no significant overall effect on weight gain, feed intake or feed conversion ratio. However, in week 1, **DMFI** was significantly ($P<0.01$) lower for **DM179** pigs. As a result the growth rate of **DM179** pigs in week 1 was significantly ($P<0.01$) lower than for **DM255** pigs. In week 1 food intake and daily gain was reduced as the dry matter concentration decreased, this trend was reversed in week 4. However, the trend was not reflected in the overall weight gain and food intake, as shown by the significant difference ($P<0.05$) in weight gain between treatments **DM255** and **DM224**. The reason for this disturbance in the trend may have been due to an unexplained result for weight gain in week 3, treatment **DM224**, which was significantly less ($P<0.01$) than for the other treatments.

3.7.3 Water usage and effluent production

There were significant differences in the total water usage of pigs on the four treatments in weeks 2, 3, 4 and overall ($P<0.01$). As a consequence of lower **DM** content of the diet the total water usage of the pigs was increased. This had the effect of increasing the volume of effluent by 88% for the **DM149** treatment compared with **DM255**.

3.7.4 Microbiology of the liquid feed system

The microbiology of the liquid feed system was studied for all four treatments. The changes in $\log_{10}$ numbers of coliforms, lactic acid bacteria, and total organisms with time are presented in figures 3.4 a,b,c and d. For all treatments the number of organisms in the feed increased rapidly over the first five days and thereafter remained relatively stable. There was very little observed difference between treatments in the numbers or pattern of colonisation of total organisms and total lactic acid bacteria. Coliforms were present in low numbers for the first three days in all treatments but almost eliminated over the 28-day trial for treatments **DM255**, and **DM149**.

Figure 3.4 Changes in the total number of organisms and in the numbers of lactic acid bacteria and coliforms in the liquid feed system at two different dry matter concentrations.

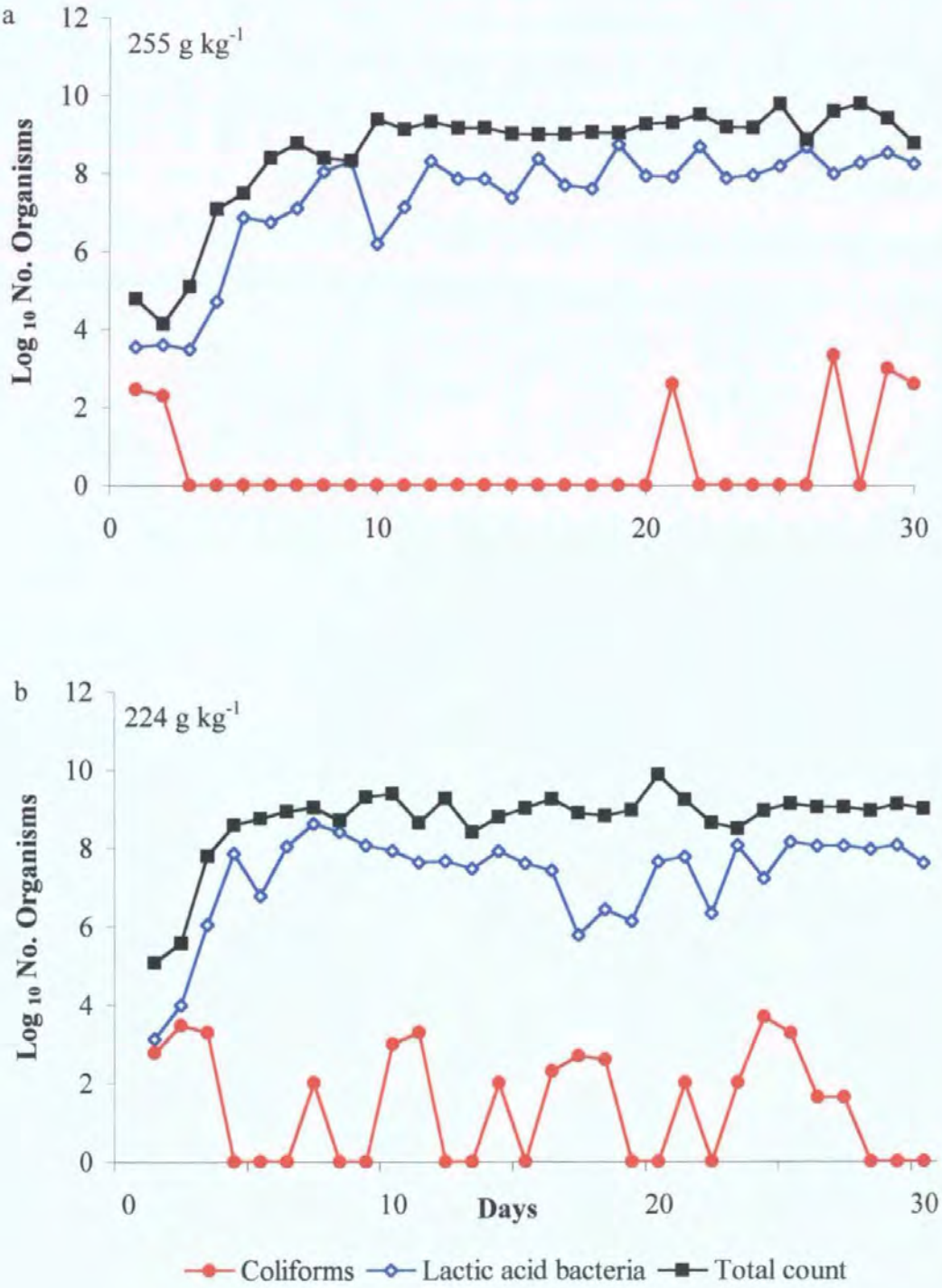
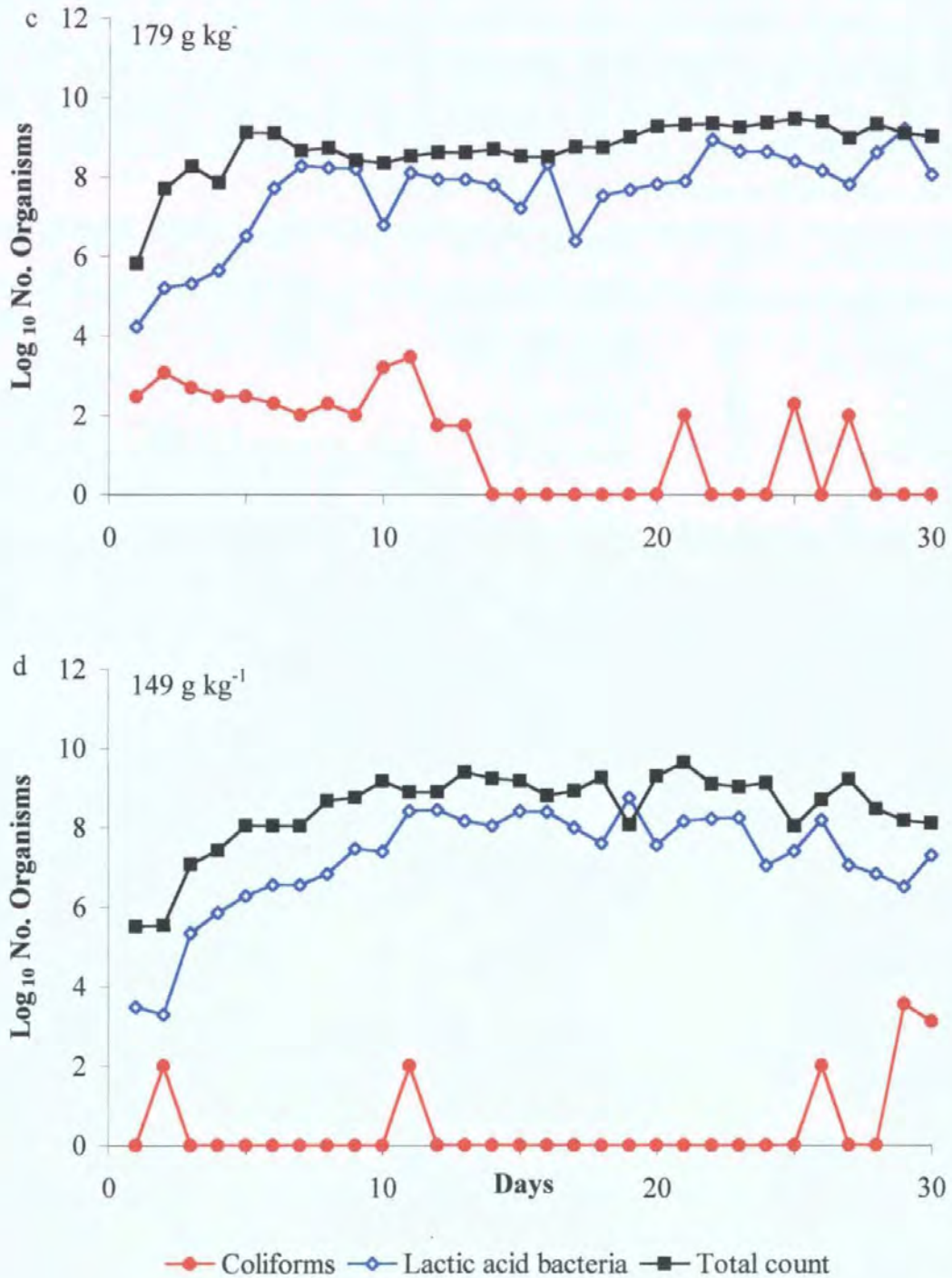


Figure 3.4 Changes in the total number of organisms and in the numbers of lactic acid bacteria and coliforms in the liquid feed system at two different dry matter concentrations.



However coliform numbers were intermittently present in appreciable numbers in **DM224** and **DM179** throughout the 28-day trial period. The relationships between lactic acid bacteria numbers and pH and between coliform numbers and pH of the feed followed a similar pattern as described in Experiment 1. The increase in lactic acid bacteria numbers resulted in a lowering of pH from 5.9 on Day 1 to 4.2 ± 0.14; 4.2 ± 0.16; 4.2 ± 0.10 and 4.1 ± 0.12 after Day 5, for the four dietary treatments respectively. The result of this was a lower number of coliforms in **DM255** and **DM149**, but this did not appear to be sufficient to suppress the growth of coliforms in treatments **DM224** and **DM179**.

3.7.5 Temperature

Temperature was recorded in three of the liquid feed system tanks. Temperature increased *circa* 6°C from 20°C to 26°C during the period of the trial.

3.8 Discussion and Conclusions

In commercial practice the weaning of piglets takes place at around 3 to 5 weeks of age. Under natural circumstances weaning would be a gradual process. Unfortunately, the economic constraints of management systems mean that it is usually a rapid process on commercial farms. The young piglets are expected to make an abrupt change from warm liquid milk delivered at approximately 50 minute intervals by the sow, to a dry diet which is available at all times.

Prior to weaning the piglets will have consumed very little dry food, and may take some time to realise that it is meant to replace sow's milk. Consequently, they may go for long periods without eating, followed by periods of gorging. Newly weaned pigs may become severely dehydrated.

Gill (1989) found that the actual consumption of water per kg body weight in the 24 hours following weaning was only proportionally 0.37 of that in the 24 hours prior to weaning and that it took more than a week for intakes of water to be restored to pre-weaning levels.

The young piglet has neither a full complement of enzyme activity nor the capability to make adult quantities of stomach acid (Partridge and Gill 1993). Consequently, when the gut is loaded with dry food the stomach pH rises and this in turn allows potentially harmful coliform bacteria to proliferate. This can cause diarrhoea which reduces performance. If the young piglets also fail to find the drinkers within 24 hours severe dehydration may occur. All of these factors can lead to reduced performance.

In an attempt to overcome these problems increasing numbers of commercial producers are turning to liquid feeding with varying degrees of success. Liquid feed is well accepted by young pigs and has been shown to enhance gut health and function by providing appropriate conditions for enzyme activity, digestion, nutrient absorption and microbial growth (Partridge and Gill 1993). Presenting the feed in liquid form may also help to overcome the problems of dehydration. The range of water to feed ratios presently being used varies greatly, and commercial producers claim that dry matter makes little difference to performance. This view is supported by some of the findings of this experiment.

Over the entire four week trial period dry matter content in the range **255** to **149** g kg^{-1} had no significant effect on dry matter intake or feed conversion ratio. However, there were differences between treatments within the period. There was a significant difference ($P<0.05$) in **DMFI** in the first week post weaning, with intake decreasing, as the **DM** content of the diet decreased. At **DM149** intake appeared to increase. However, visual observation of the pens together with the poorer growth rates, and higher feed conversion

ratios, suggested that the apparent increased intake may have been due to greater feed wastage on this treatment. Weight gain decreased significantly ($P<0.01$), during the first week post weaning as the dry matter content of the diet was reduced. Consequently, the feed conversion ratio increased as the **DM** content decreased ($P<0.05$) for the first week post weaning. Conversely, pigs fed on the most dilute feed (**DM149**) had the highest daily live weight gain in the final week of the trial (543 vs 449 $\pm$ 42 g d^{-1} for those on **DM255**). Thus although piglets on the most dilute diets suffered the greatest post weaning growth check, they were able to compensate for the loss and attain equally good weights at the end of the 28 day trial.

The live weight of the pig and hence the estimate of live weight gain could have been influenced to a small extent by differences in gut size and/or gut fill arising from the different diets. Incorporation of more bulky or fibrous components into the diets of pigs to reduce diet density has been shown to increase the weight of gut components and through this to have a significant effect on the killing out percentage of finishing pigs (Cole, *et al.* 1968b; Cole and Chadd 1989; Low 1993). Although liquid diets may appear to have more 'bulk' than dry feeds, adding water to the diet does not appear to increase gut size in the way that fibrous components do. Thus pig ration-fed diets in either a dry meal or liquid form had similar killing out percentages (Smith 1976; Patterson 1989a; Patterson 1989b). This is not surprising, as studies by Barber (1992) and Gill (1989) have shown that generally it is voluntary water intake which limits dry matter intake rather than the reverse. Hence the total volumetric intake will be comparable when the same diet is fed in liquid or dry form. Where comparisons have been made of finishing pigs which were ration fed, or fed *ad libitum* the latter have had lower killing out percentages (Cole *et al.* 1968b; Patterson 1989b). This implies that when pigs are fed *ad libitum* they are able to overcome limits imposed by stomach capacity by altering their feeding behaviour.

Furthermore, Low (1993) has pointed out that the liquid and solid phases of the digesta move at different speeds in the gut. Consequently, the greater volume of a liquid diet may have only a transient effect on gut fill and little long term effect on the development and ultimate size of the gut.

The dilution of feed did have a noticeable effect on the total volumetric intake of the piglets, and on voluntary water intake from the drinkers as illustrated in Figure 3.5. The findings of this experiment would support the theory of Yang *et al.* (1981) who suggested that the pig will limit water intake in order to maximise food intake. There is evidence that a pig eats to a constant daily digestible energy intake and, within limits, is able to compensate for variation in nutrient density of the diet by changes in feed intake (Cole and Chadd 1989). The mechanism whereby the pig attempts to adjust its daily intake by eating less of high-energy diets, and more of low energy diets has been described by (Cole *et al.* 1972). These workers hypothesised that this mechanism worked adequately up to a point, beyond which physical limitation prevented further intake of digestible energy. The results obtained in this experiment suggest that increased fluid content of the diet does not produce such a physical limitation.

In Experiment 2, the pig's response to increasing dilution of the diet appeared to be biphasic. There was no difference in DM intake overall between treatments. Compared with the pigs on the highest dry matter diet (**DM255**) the pigs on **DM224** reduced their intake of water from the drinkers to maintain a similar DE and total volumetric intake as illustrated in figure 3.5. At lower DM concentrations (**DM179** and **DM149**), the pigs maintained their DM intake by increasing their total volumetric intake as illustrated in figure 3.6.

Figure 3.5 Components of total volumetric intake of pigs fed four different dry matter concentrations.

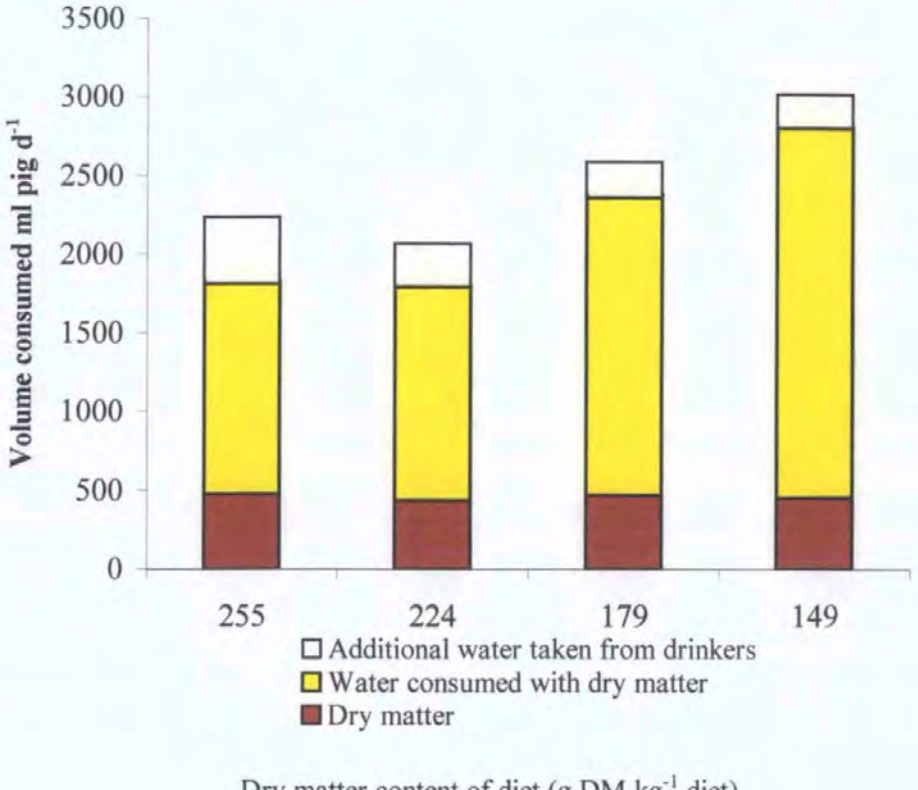
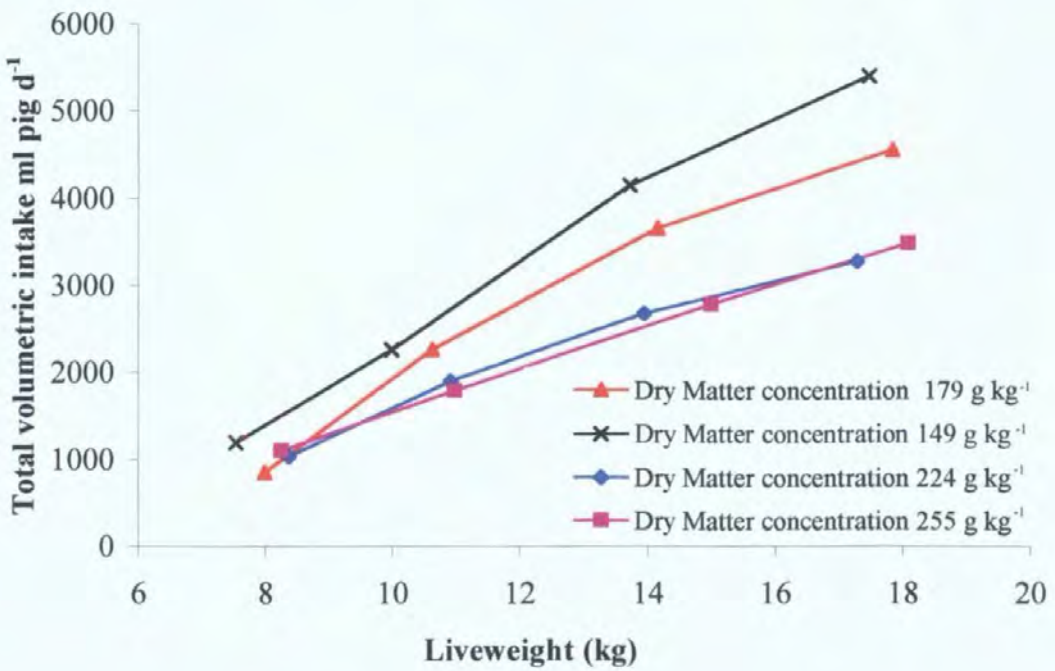


Figure 3.6 The relationship between total volumetric intake and liveweight of weaner pigs.



There is evidence from the current study that weaner piglets have a minimal requirement for water taken separately from liquid feed. Even though it meant increasing their volumetric intake, the piglets continued to consume water at an average of 223 ml d^{-1} from the nipple waterers even at the lowest DM concentration. This water usage is probably a behavioural obligation as the liquid present in the most dilute diets would have been more than adequate to meet their requirements (Brooks and Carpenter 1993).

Yang *et al.* (1981) concluded that the total volumetric intake of pigs in the weight range (about 30 kg) was 19% of live weight, whereas Barber (1992) concluded that volumetric intake was only 12% of live weight in heavier pigs (30 - 60 kg). Both these estimates were based on pigs fed dry diets and given access to a separate water supply. The pigs used in this experiment were lighter (average 8 - 17 kg) than those used by Yang or Barber and were fed liquid diets. On **DM149** total volumetric intake was 30% of live weight. This suggests that volumetric intake per unit live weight decreases as the pig grows which would be consistent with the changing ratio of stomach volume to carcass weight. On both **DM255** and **DM224** total volumetric intake represented 19% of the liveweight and would seem to represent the level at which the pig can still assert some control over volumetric intake.

The point at which further increases in the dilution of the diet will start to increase effluent production can be calculated from the results obtained in this experiment. It would appear that the pigs had an obligatory water demand of 223 ml d^{-1} (mean value of the two most dilute diets), and that their DM requirements was 456 g d^{-1} (mean value overall). The pigs appeared to be able to minimize their volumetric consumption on treatments **DM255** and **DM224** by adjusting voluntary water intake. Thus the pigs' desired volumetric intake can be estimated as 2256 ml d^{-1} and its DM intake and obligatory water intake are 456 and 223

g d^{-1} respectively, thus the quantity of water that can be fed with the dry matter without resulting in an unnecessary increase in effluent production would be 2256 - (456+223) = 1577 ml d^{-1}. This would be equivalent to a water to feed ratio of approximately 3.5:1, (220 g DM kg^{-1}). In summary it can be concluded that the performance of weaner pigs fed a liquid fermented diet *ad libitum* on a range of dry matter concentrations was excellent and that dilution made little difference to the overall performance of the piglets.

CHAPTER 4

THE USE OF INOCULANTS TO CONTROL FERMENTATION IN LIQUID DIETS FOR WEANER PIGS

General Introduction

Experiments 1 and 2 established that weaner pigs can grow better on liquid feed than dry and that they will accept liquid diets with a range of dry matter concentrations. The microbial examination in Experiments 1 and 2 revealed a natural fermentation and reduction in pH of the liquid feed, (*circa* pH 3.5 - 4.0), where lactic acid bacteria proliferated and became the dominant organism within five days. Either or both of these factors may have had an effect on the performance of the weaner pigs. The reduced pH of the diet may have been beneficial in maintaining the acid environment in the stomach of the newly weaned pigs and may have been helpful in preventing colonisation by undesirable organisms (Bolduan, *et al.* 1988; Radecki, *et al.* 1988). All organisms have an optimum pH for growth and the tolerance of different species to pH levels outside their optimum varies (Banwart 1989). In most cases enteropathogenic bacteria are not very tolerant of pH values below 5.0, whilst gut microflora tend to favour a more acid environment (Kershaw *et al.* 1966; Banwart 1989).

Suckling piglets have an intrinsic mechanism which helps to maintain a healthy gut environment and inhibit the growth of pathogenic bacteria whilst allowing the commensal bacteria to flourish (Tannock 1990; Maxwell and Stewart 1995). This mechanism depends mainly on the production of acid. In the suckling piglet gastric secretion of hydrochloric acid is very limited for the first few weeks of life and sow's milk does not provide a strong stimulus (Kidder and Manners 1978). The piglet is able to overcome the disadvantages of having low HCl production by fermentation of the lactose in sow's milk to lactic acid, which occurs in the stomach as a result of microbial fermentation. Cranwell, Noakes and

Hill (1968) have shown an inverse relationship between lactic acid production and hydrochloric acid secretion in suckled piglets. Their interpretation is that the acidity produced by lactic acid from fermentation in the stomach of suckled piglets could be sufficient to suppress hydrochloric acid secretion so that the pH remains high enough for rapid proliferation of *lactobacilli* but not other organisms. They found that this effect was observed where pigs were reared under 'conventional' farm conditions, where bacterial exposure was likely. *Lactobacilli*, strains of which are naturally occurring intestinal bacteria, are present on the teats of the sow and will be ingested when the piglets suckled the sow (Close 1993). In a recent study on the University farm, *Lactobacillus fermentum* and *Lactobacillus acidophilus* were recovered from the teats of sows which had just farrowed (Santos 1996).

The problem of 3 and 4 day scouring, and post weaning growth check, occurs when the mechanism fails. This is because the suckling pigs have been removed from the sow and hence from the supply of lactose, and lactic acid bacteria. There exists a critical point immediately after weaning when numbers of *lactobacilli* decline and pathogenic bacteria increase (Huis in't Veld and Havenaar 1993).

Manufacturers of compound feeds add acids to dry feeds in order to overcome the acid binding capacity of some feed ingredients, to increase proteolysis and to reduce scour incidence (Easter 1987; Bolduan *et al.* 1988). The addition of lactic acid, fumaric acid, formic acid, and citric acid to the feed have all been shown to improve growth rate and feed efficiency in pigs (Falkowski and Aherne 1984; Edmonds *et al.* 1985; Geisting and Easter 1985; Bolduan *et al.* 1988; Risley *et al.* 1991; Risley *et al.* 1993; Roth *et al.* 1993; Krause *et al.* 1994). In pigs, colibacillosis has been prevented by the addition of lactic acid to the drinking water (Kershaw *et al.* 1966; Thomlinson and Lawrence 1981).

In recent years there has been considerable interest in the possibility of using probiotics instead of antibiotics in piglet diets (Pollman 1986; Ewing and Haresign 1989). The probiotic virtues of lactic acid bacteria have been acknowledged for a long time (Bottazzi 1983; Fuller 1992a; Ray and Daeschel 1992). *Lactobacilli*, Enterococci, and Bifidobacteria are commonly used as probiotics for pigs (Jonson and Hemmingsson 1991; Ewing and Haresign 1989). In Experiments 1 and 2, the high levels of lactic acid bacteria could have exerted a probiotic effect and the presence or absence of microbial activity may be an important determinant of success in the liquid feeding of weaners.

In the human food processing industry lactic acid bacteria or 'starter cultures' are used to ferment foods in order to preserve them (Dillon and Cook 1994). *Lactobacillus helveticus* and *Lactobacillus delbreukii* are the most commonly used as lactic starter cultures. Several thousand fermented foods are prepared from milk, meat, fish, vegetables, grains, lentils and fruits (Ray and Daeschel 1992; Pederson 1979; Wood 1985; Rose 1982; Campbell-Platt 1987; Gilliland 1985). The fermentation in some of these products is initiated by the addition of a starter culture, as either single or mixed strains, generally used at a level which provides 10^6 cells ml^{-1} of raw material (Gilliland 1985; Ray and Daeschel 1992). Depending on the strains used the starter cultures can produce many types of anti-microbial compounds including organic acids, alcohols, diacetyl, H_2O_2, reuterin and bacteriocins (Gilliland 1985; Ray and Daeschel 1992; Ray and Daeschel 1994; Dillon and Cook 1994; Yang and Ray 1994). In theory the technology used for processing human foods could be adapted for use in liquid feed system for pigs, either by adding starter cultures to compound feeds mixed with water, or raw materials destined for use in liquid feed systems, or to food industry liquid residues (FILR) destined for use in pig diets.

Pig producers utilize a wide range of FILR from the human food and drink processing

industries in liquid feeding systems. These FILR include milk by-products, starch residues and brewery waste, potato processing waste, grain processing wastes sugar industry wastes, fruit and vegetable processing wastes and fish silage (Brooks and McGill 1995). In the Netherlands, the disposal of FILR would create a serious environmental problem if they were not used to feed to pigs. The pig industry in the Netherlands feeds vast quantities of FILR to pigs through liquid feeding systems.

Many of these FILR are colonized by different microorganisms. For example, whey, C-Starch and brewery wastes, are likely to support populations of bacteria and yeasts depending on the processing involved (Perry 1995). Virtually all FILR will provide a substrate capable of contamination by spoilage organisms unless such contamination is prevented or controlled (Pederson 1979). The use of these types of FILR produces variability in their pattern of fermentation. This is because they all contain different populations of microorganisms and are mixed together in varying proportions on the farm according to availability of the product (P. McTiffin personal communication 1996).

Strains of *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus fermentum*, *Lactobacillus caesei* and *Pediococcus acidilactici* have all been used as inoculants to control fermentation in human foods such as, soda crackers, fermented milks and sausages (Bottazzi 1983; Gilliland 1985; Campbell-Platt 1987; Ray and Daeschel 1992).

If the fermentation pattern of liquid feed could be successfully controlled by the use of such bacterial inoculants, then the risk of coliform scours developing post weaning could be reduced. This in turn would improve the growth rate and feed efficiency of weaned piglets. The use of bacterial inoculants which would lower the pH of the diet could reduce the need to add expensive organic acids and/or antibiotics to piglet diets which are destined for use

in liquid feed systems. However, more information is needed before organic acids and antibiotics can safely be withdrawn from pig feeds.

The main objectives of the experiments reported here were:

- to compare fermentation patterns in diets with and without the addition of inoculants
- to compare the growth performance, feed efficiency and health of newly weaned piglets fed liquid diets in which pH was reduced either by the addition of lactic acid or through acidification by lactic acid bacteria
- to examine changes in the microflora of diets over time
- to examine changes in the composition and nutritional value of the diets as a result of fermentation or acidification

EXPERIMENT 3 A COMPARISON OF THE FERMENTATION PATTERNS IN A LIQUID DIET WITH AND WITHOUT THE ADDITION OF 'PRONIFER".

4.1 Introduction

Pronifer (Produktionsgenmeinschaft, Austria) is a commercially available feed additive derived from a specific lactic acid fermentation of heat-treated soyabean meal and malt, by a mixture of *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus fermentum*, *Lactobacillus caesei* and *Pediococcus acidilactici*. The manufacturers describe it as a 'natural feed additive' which imparts the benefits of maintaining the health of piglets. The addition of Pronifer to piglet diets was found to improve the gut flora balance, produce less scours at weaning and exert a positive effect on certain immunological parameters. Results have also indicated that weight gain and feed conversion ratio was improved (Manufacturers Technical bulletin 1994). The purpose of this study was to evaluate whether the inoculant "Pronifer" was a suitable candidate to control the fermentation in the liquid feed system for weaner pigs.

4.2 Materials and Methods

4.2.1 Experimental treatments and diet

The experiment consisted of two treatments.

NP Control liquid diet in which natural fermentation was allowed to take place in tank 1

PRO Liquid diet to which a single inoculant of one litre cultured Pronifer was added to tank 2.

The liquid diet comprised 100 l of water to which 40 kg of a commercial diet (Roche Products Ltd, Heanor, Derbyshire) was added to give a dry matter concentration of 255g kg^{-1}. The declared nutrient composition of the diets has given previously (Chapter 3, Table 3.4).

4.2.2 Preparation of pre-culture of Pronifer

The pre-culture of Pronifer was prepared according to the manufacturers recommendations. 400 g of feed grade, defatted, soyabean meal and 150 g of skim milk powder were mixed with 10 l of water in a glass container. To this substrate was added one litre of Pronifer-MSB paste. The pre-culture was incubated aerobically at 38°C for 8 hours.

4.2.3 Trial procedure

The trial was conducted using the feed tanks described in Chapter 2, 2.1.1. Before commencement of the trial the tanks and system were cleaned using hypochlorite solution as described in Chapter 2, 2.1.2).

Feed and water were added to the mixing tanks and remained within the tanks for 10 days. One litre of the Pronifer pre-culture inoculant was added to tank 2 immediately following mixing of the feed and water and the automatic pumping system was set in operation to ensure thorough mixing of the feed.

Each morning at 9.30 hours the tanks were mixed for two minutes using the automatic pumping system. 100 ml samples of the feed mix were removed immediately after mixing from each tank *via* the outfall pipe, using aseptic procedures. A microbial assessment was conducted on samples from both treatments as described in Chapter 2, 2.2.1, with the exception that all samples were plated within one hour of collection. Additionally on day

2, 4 and 7 a further 100 ml sample of the liquid feed was withdrawn from both tanks. This sample was used to assess changes in the proportions of different sugars using HPLC as described in Chapter 2, 2.2.4. These samples were analyzed within one hour of collection. The liquid feed was filtered through filter paper (Whatman Inercem Ltd, Maidstone, U.K.) and 20 µl sample of the filtrate was injected into the HPLC for analysis.

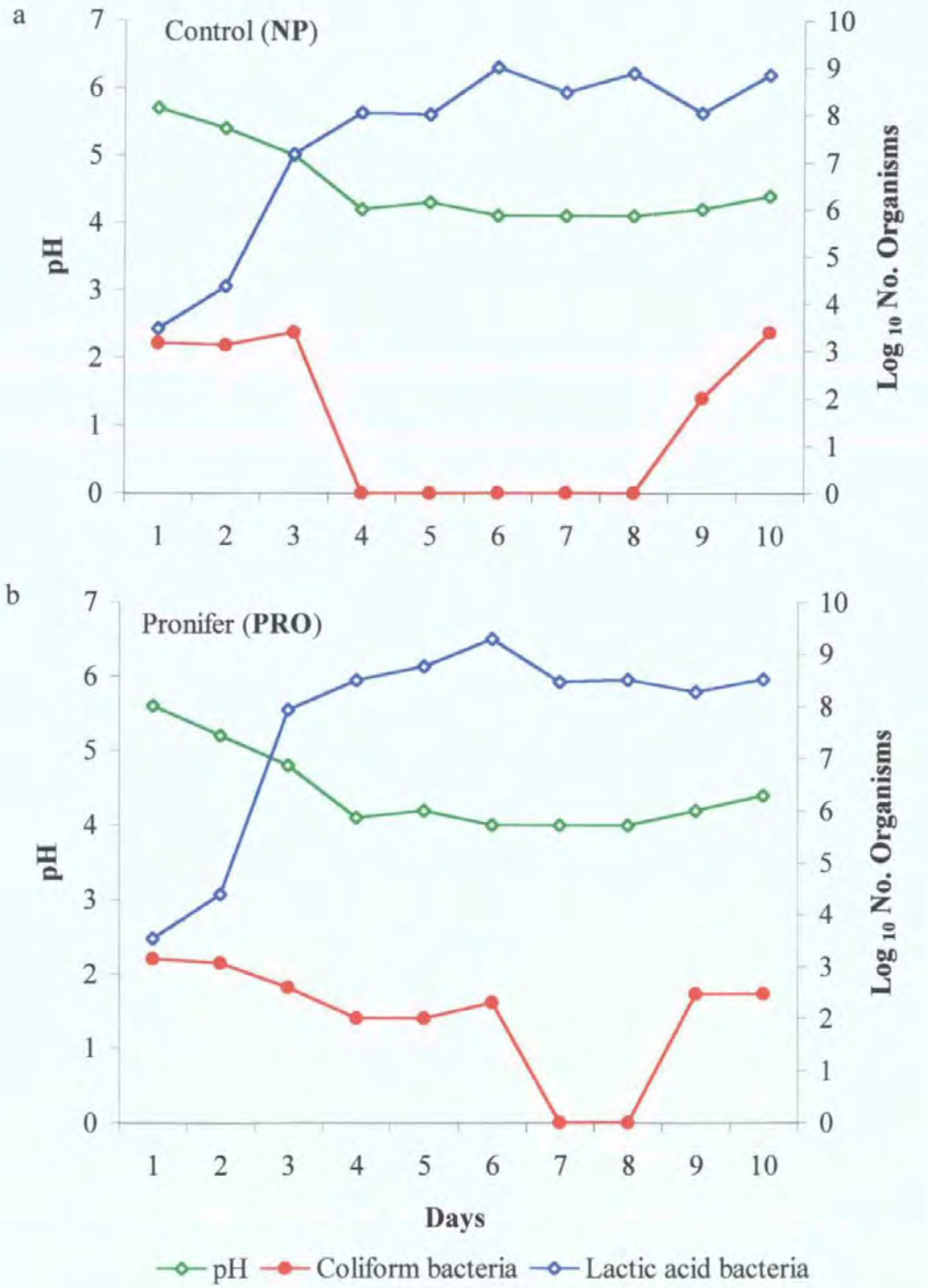
The temperature of the liquid feed tanks was monitored using a Tinytalk-Temp as described Chapter 2, 2.2.5.

4.3 Results

4.3.1 Microbiology of the liquid feed system

The changes in $\log_{10}$ numbers of coliforms and lactic acid bacteria on pH with time are presented in figure 4.1 a,b. For both treatments the number of lactic acid bacteria increased rapidly over the first four days and thereafter remained relatively stable. There was very little difference between treatments in the numbers or pattern of colonisation of total lactic acid bacteria. There was a difference in the pattern of colonisation of coliforms between treatments. Coliforms were present in low numbers for the first 3 days in **NP** and eliminated for a period of 5 days before reappearing at similar levels for the last 2 days of the experiment. However, coliforms were present in low numbers for most of the time in treatment **PRO**. The increase in lactic acid bacteria numbers resulted in a lowering of pH from 5.6 and 5.7 on day 1 to pH 4.4 on day 10 for both **PRO** and **NP** treatments. This pH resulted in a lower number of coliforms in **NP** treatment but did not appear to be sufficient to suppress the growth of coliforms in the **PRO** treatment.

Figure 4.1 The relationship between lactic acid bacteria, coliform numbers and pH in the liquid feed system with time.



4.3.2 Temperature

The temperature of the liquid feed contained in both tanks was recorded and the relationship between results $\log_{10}$ numbers of lactic acid bacteria and temperature are presented in figures 4.2 a,b. As the population of lactic acid bacteria increased the temperature of the liquid feed was increased by 9.1°C and 6.6°C for treatments **PRO** and **NP** respectively.

4.3.3 Changes in sugars in the liquid feed

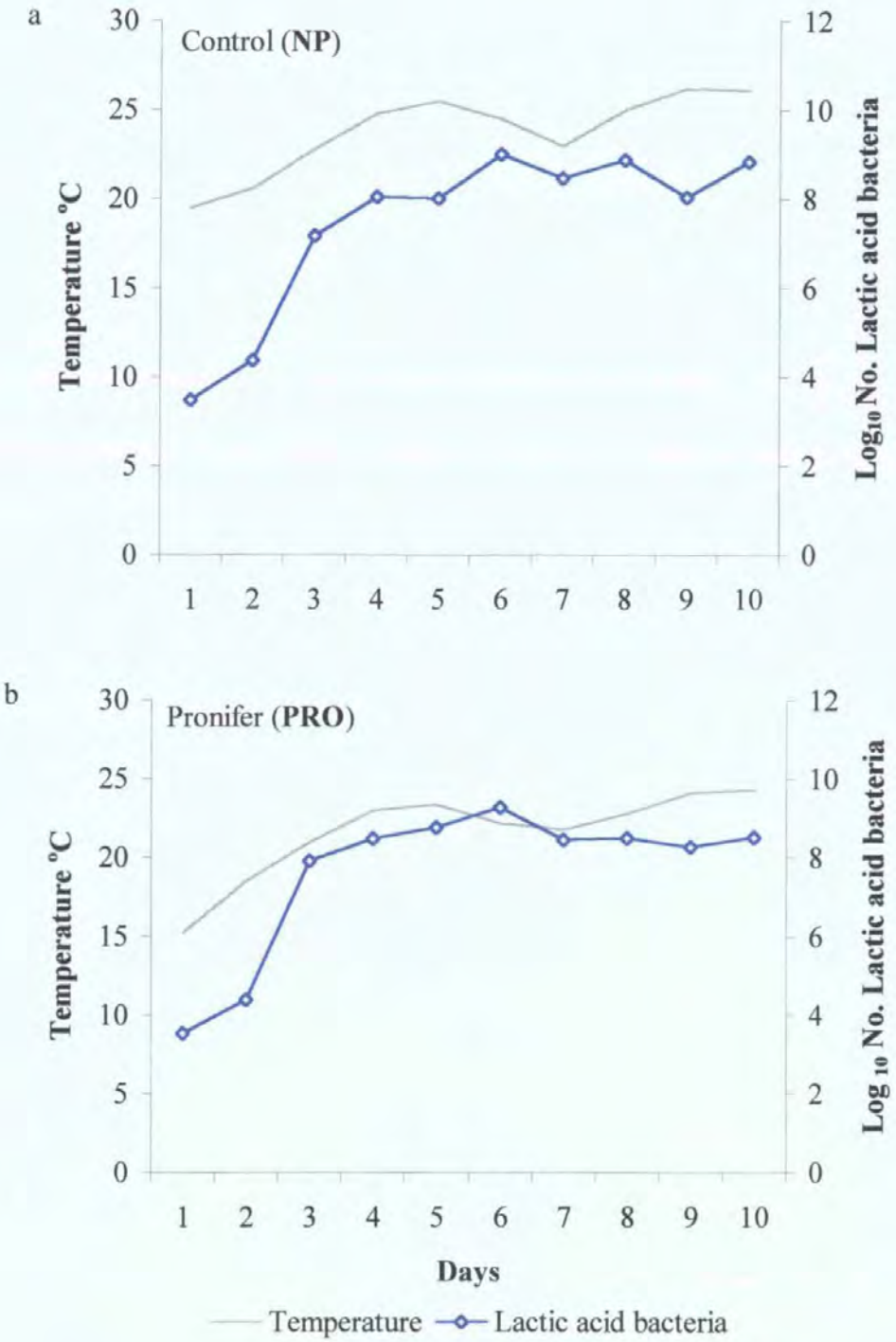
The four sugars which were identified as being present in the liquid feed system were; glucose, sucrose, maltose and lactose. The most significant change was that of glucose, which was present on day 1 for both treatments, decreased rapidly over 2 days for **NP** but more slowly over 7 days for **PRO**. There was very little change in any of the other sugars as a result of fermentation.

4.4 Discussion and Conclusions

Pronifer did not produce a fermentation pattern or pH significantly different from **NP**. Furthermore, the pH did not fall below pH 4.0. As Pronifer did not produce a rapid drop in pH and subsequent decrease in coliform numbers it would not appear to be a particularity suitable inoculant for liquid feed systems. However, gut colonisation was not studied in this experiment and there could have been differential and beneficial differences in colonisation.

The temperature of the liquid feed increased in parallel with the increase in the population of lactic acid bacteria. It was observed that the temperature increase was greater for **PRO**, which may imply that these bacteria have a higher metabolic rate during their growth phase than the naturally occurring bacteria found in **NP**.

Figure 4.2 The relationship between microbial activity and temperature in the liquid feed system with time.



This increase in temperature may be an advantage in that warm liquid feed may be more acceptable to young piglets than cold liquid feed. Increases in temperature also affect the rate of natural enzyme activity, depending on their optimum operating temperatures, and it may be expected that any changes in the breakdown of sugars would increase as temperature increases, until the optimum for enzyme activity has been reached.

Both treatments had very similar patterns of lactic acid bacteria colonisation, however, it was observed that there was a greater reduction of coliform numbers for treatment **NP** (Figure 4.1 a,b.). This may imply that factors other than pH may be responsible for the reduction of coliform numbers in the liquid feed.

The results suggest that many interactions were taking place between the components of the system such as, water, oxygen, yeasts and bacteria, which were present in the liquid feed system. Fermentation is a chemical action brought about by bacteria or yeasts. Yeasts are widely distributed on the skins of fruit and vegetables and are all capable of breaking down glucose, sucrose, maltose and lactose (Bouix and Leveau 1995). The living yeast cell produce natural enzymes which operate at an optimum temperature of 25°C - 30°C. The yeasts present would have produced ethanol and carbon dioxide. In this experiment it was found that the proportion of glucose decreased over time. Glucose, being a prime substrate for the generation of energy, is utilised readily by yeasts as well as by lactic acid bacteria. In this experiment it was found that the time at which most sugars disappeared coincided with the optimum temperature for yeast activity at about day 5. If sugars are provided in concentrations which are too high then alcohol might be produced at a level which may be detrimental to weaner pigs.

Lactic acid bacteria metabolise glucose and lactose whether they are homo-fermentative or

hetero-fermentative bacteria. However, lactic acid bacteria will utilize glucose in preference to other sugars because it is in a form which requires less energy to assimilate (Stryer 1988). This would explain the rapid decrease in glucose in this experiment.

To ensure that the lactic acid fermentation works efficiently in the liquid feed system the inoculated lactic acid bacteria need to be provided with a suitable carbohydrate substrate. This should contain sugars, such as glucose, sucrose and maltose to provide an energy source which satisfies the specific requirements of the bacterial inoculant used. If the aim is to ferment the diet, nutritionists may need to adjust the nutrient specification of liquid diets in order to encourage an efficient fermentation.

EXPERIMENT 4 EFFECT ON WEANER PERFORMANCE AND DIET MICROBIOLOGY OF FEEDING A LIQUID DIET ACIDIFIED TO pH 4.0 WITH LACTIC ACID OR THROUGH FERMENTATION WITH *pediococcus acidilactici* .

4.5 Introduction

Diets for young pigs are often acidified using organic acids such as formic, fumaric, propionic, lactic or citric acid in order to reduce post weaning disturbances and to compensate for the piglets lack of gastric secretion of hydrochloric acid (Jongbloed and Jongbloed 1995). However organic acids are expensive, corrosive, difficult to handle and transport and can be harmful to humans. An alternative to the use of organic acids to control pH and reduce post weaning disturbances is to use an inoculant which will bring about a lactic acid fermentation. There are several inoculants which are commercially available for use in pig diets and these could be used in liquid feed systems for weaner pigs. Bactocell is one of these products.

Bactocell (Park Tonks Ltd., Cambridge, UK), consists of a single live strain (MA18/5M) of *Pediococcus acidilactici* (PA). PA has been widely used in the human food processing industry, and occurs naturally on a range of fermenting animal and plant products where carbohydrates are available. It is also present in the intestinal tracts of various warm blooded animals. PA was first discovered in 1887 by Lindner (Buchanan and Gibbons 1974) and is described as being a gram positive cocci of the family Streptococcaceae which produces a homo-lactic fermentation with DL-lactic acid and diacetyl as the end products. The species *Pediococcus* will ferment a range of substrate including glucose, fructose and mannose, but more specifically PA will also ferment galactose, arabinose, xylose, salicin and trehalose. Some strains will also ferment lactose (Buchanan and Gibbons 1974). The

optimum temperature for growth is suggested as 40°C (Buchanan and Gibbons 1974; Ray and Daeschel 1992) but growth can occur at 50°C (Leveau, Bouix and Roissart 1995). The only other *Pediococcus* strain to grow at 44°C is *Pediococcus pentosaceus* (Buchanan and Gibbons 1974; Ray and Daeschel 1992). Leveau *et al.* (1995) suggested that there does not appear to be any culture medium for the specific isolation and enumeration of these bacteria.

Antibacterial bacteriocins produced by many strains of lactic acid bacteria have generated interest as potential food bio-preservatives (Ray and Daeschel 1994). They are bactericidal to many bacteria associated with food spoilage and food-borne illnesses (Biswas, Ray, Johnson and Ray 1991). Bacteriocins have been consumed by humans for thousands of years through fermented foods without any known adverse effect (Yang and Ray 1994). Some strains of PA are known to produce pediocins which are bacteriocins which have been shown to inhibit *Listeria monocytogenes* (Pucci, Vedamuthu, Kunka and Vandenbergh 1988), and are thought to be suitable for use as food biopreservatives (Biswas *et al.* 1991). PA also produces diacetyl which is antibacterial against gram negative bacteria, yeasts, and mould. Sensitivity of these organisms to diacetyl has been shown to increase when the pH is reduced to 5.0 (Jay 1982).

Juven, Meinersmann and Stern (1991) reviewed the use of Pediococci to control enteropathogens in poultry destined for human consumption and found them to be suitable candidates because they were known to produce antagonistic substances found to be active against a broad spectrum of bacterial species including human pathogens.

Pediococcus acidilactici could, in theory, be a useful candidate for the control of fermentation of liquid diets for pigs and has the potential to provide a degree of biosecurity

in such systems. This experiment examined the effect on performance of weaner piglets of reducing the pH of a liquid diet either with an organic acid or with a lactic acid bacterial inoculant. In the control diet pH was reduced to 4.0 by the addition of an organic acid and in the other treatment a similar pH was achieved through the use of *Pediococcus acidilactici* a microbial inoculant.

4.6 Materials and Methods

4.6.1 Acceptability of the diets to newly weaned piglets

Before undertaking the main feeding trial the acceptability to piglets of diets which had been fermented with (PA) or treated with lactic acid was tested before the main feeding trial commenced. Piglets were offered one of two dietary treatments, fed by hand, which were prepared as follows:

LA The **LA** liquid diet was prepared by mixing 4 kg of Diet 1 with 9,824 ml of water and 176 ml of DL-lactic acid in a 20 l plastic bucket to provide a DM concentration of 255 g kg^{-1} (water feed ratio of 2.5:1). The resultant liquid diet which had a pH of 4.0 was allowed to soak for 3 days before feeding to the piglets.

PA The **PA** liquid diet was prepared as for **LA** except that lactic acid was replaced by an inoculation of 0.20 g of *Pediococcus acidilactici*.

4.6.2 Diets

The diets used were commercial diets manufactured by A-One Feed Supplements Ltd. (Thirsk, North Yorkshire). The declared nutrient composition of the diets is given in table 4.1.

Table 4.1 Declared nutrient composition of the diets used in Experiment 4.

	Diet 1	Diet 2
Digestible energy (MJ DE kg^{-1})	16.6	16.2
Crude protein (g kg^{-1})	220	225
Crude fibre (g kg^{-1})	23	25
Total ash (g kg^{-1})	55	55
Oil (g kg^{-1})	65	80
Lysine (g kg^{-1})	17	16
Vitamin A (iu kg)	15,000	15,000
Vitamin D3 (iu kg^{-1})	2,000	2,000
Vitamin E (iu kg^{-1})	250	250
Avilamycin [(a)] (mg kg^{-1})	40	40
Copper sulphate (mg kg^{-1})	175	175

[a] Avilamycin (Maxus, Elanco Products Ltd, Basingstoke, Hampshire)

Diet 1 contained 45% cooked cereals in the form of oats and maize, in a ratio of approximately 1:1. Milk products (primarily skim milk and whey powder) contributed 15% lactose in the final diet. In addition glucose was added to provide 2.5% of the final diet. Protein and fat were supplied as steam dried fish meal (68 - 70% protein; 10 - 12% oil) and full fat soya bean oil. In Diet 2 the cereal component contained 49.5% of cooked cereals in the form of maize and wheat, in a ratio of approximately 1:1. The main milk product was skim milk powder with some whey powder; together these contributed 10% lactose in the final diet. In addition glucose was added to provide 2.5% of the final diet. Protein and fat were supplied as steam dried fish meal (68 - 70% protein; 10 - 12% oil) and full fat soya bean oil. Both diets were supplemented with minerals, trace elements and vitamins.

4.6.3. Housing and Feeding

The diets were offered to two male and two female weaner piglets, Large White x (Large White x Landrace), Camborough hybrids, Pig Improvement Company, (Fyfield, Wick), of similar age (24 ± 3 days) and with an average weight of 7.6 kg. One male and one female

pig were housed in each of 2 pens which were maintained within the pigs thermoneutral zone by electric heat lamps in the pens and electric fan heating in the building. Piglets in pen 1 were offered (**LA**), and piglets in pen 2 were offered (**PA**). The food and water were supplied in separate troughs and piglets were fed twice daily, by hand. The quantity of food and water consumed was measured by weight using an electric balance. The health and behaviour of the pigs was observed closely for the period of treatment, and any veterinary interventions were recorded.

4..6.4 Results of preliminary feed trial

The results of this feed acceptability study are summarised in table 4.2.

Table 4.2 Food and water intake of pigs fed a liquid diet containing lactic acid or *Pediococcus acidliactici.*

Treatment	Liquid feed intake ml pig d^{-1}	Water intake ml pig d^{-1}	Total volumetric intake ml pig d^{-1}
LA	757	47	804
PA	471	164	635

Piglets fed the **LA** diet began eating on the first day and maintained a healthy appetite and condition throughout. Feed intake was comparable with those of pigs fed a liquid diet of similar dry matter concentration in Experiment 2.

The male piglet on treatment **PA** appeared stressed on weaning and developed a swollen shoulder which required veterinary intervention (1ml injection of long acting Duphapen, Solvay Duphar Veterinary, Southampton). As a result of this ill health, (which was not considered dietary related), the pig had a decreased appetite and was drinking more than expected. Upon recovery from the illness it began to eat and drink normally. However, the female on treatment **PA** began eating after the first day and maintained a healthy

appetite and condition throughout the trial period. As a result of this preliminary study the liquid diets were considered to be suitably palatable and a decision was taken to proceed with a full scale feeding trial.

4.7 Feeding Trial

4.7.1 Experimental facilities

These have been previously described in Chapter 2, 2.1.

4.7.2 Experimental design and treatments

Forty-eight Large White x (Large White x Landrace) weaner pigs (Camborough hybrids, Pig Improvement Company (Fyfield, Wick)), average weight (7 ± 1 kg) and age (24 ± 4 days), were randomly allocated to 8 pen groups comprising six piglets each. This was to compare the effect of feeding weaner pigs *ad libitum* liquid diets either inoculated with **PA** or acidified with **LA**. The two dietary treatments were:

LA Piglets were fed *ad libitum* on a commercial meal, early weaner diet mixed with water to provide a dry matter concentration of 255 g kg^{-1} (water feed ratio of 2.5:1) with the addition of DL-lactic acid (Ellis and Everard, Exeter), adjusted to a pH of 4.0. Lactic acid was added with each new batch of feed, at the rate of 440 ml per 10 kg of dry matter to maintain the desired pH. The acid was substituted for an equal volume of water in order to maintain the dry matter (DM) concentration. The pigs received Diet 1 for the first 14 days post weaning. The diet was changed to Diet 2 for the remainder of the trial. Residual feed in the tank ensured that the change from Diet 1 to Diet 2 was a gradual process. Feed was dispensed to the pigs automatically using an *ad libitum* feed delivery system previously described in Chapter 2, 2.1.1.

PA Piglets were fed as described for **LA** with the exception that lactic acid was omitted and *Pediococcus acidilactici* was added at the rate of one gram of *Pediococcus acidilactici* per 20 kg of dry matter. The inoculant, which was in freeze dried form, was introduced with each new batch of feed during mixing.

The treatments were replicated four times. A replicate consisted of two pen groups (12 pigs); each replicate consisted of three females and three entire male piglets. The dietary treatments were introduced at weaning and continued for 28 days.

4.7.3 Trial procedure

Piglets were weighed at weekly intervals throughout the experimental period. The health of the animals was monitored closely and all medications and veterinary interventions recorded. Feed intake, weight gain, water intake and effluent production records were maintained throughout the trial. Feed and water were introduced to the mixing/recirculating tank three days before treatment began. The liquid feed in the mixing tanks was not allowed to fall below 200 l (approximately half the volume) before replenishment. Additions of new feed were made to the liquid feed tanks on a daily basis.

The temperature of the liquid feed tanks was monitored using a Tinytalk-Temp as described in Chapter 2, 2.2.5. In addition to the liquid diet clean water was freely available to the pigs at all times from nipple waterers in the pens.

4.7.4 Sampling procedures

Prior to replenishment in the morning, samples of approximately 300 ml were withdrawn from the liquid feed mixtures using aseptic procedures after two minutes of mixing. The samples were assessed for microorganisms, changes in sugars, alcohol, and changes in

dietary composition. A microbiological assessment was made of the liquid feed mixture for each treatment. In addition a microbiological assessment was made of the viable indigenous microorganisms present in the dry diets 1 and 2 on days 1, 7, 14 and 21 of the experiment. The samples were plated within two hours of collection, using the techniques described in Chapter 2, 2.2.1.

4.7.5 Statistical Analysis

Feed intake was calculated on a dry matter food intake (**DMFI**) basis. Dry matter feed conversion ratio (**DMFCR**) was the appropriate multiple of **DMFI** divided by the weight gain of the pigs. Performance data were subjected to two way analysis of variance. Daily gain was also analysed using covariance analysis (using weaning age as the covariate). All statistical analyses were undertaken using Minitab *v* 9.2 (Minitab Inc., State College, USA 1993).

4.8 Results

4.8.1 Animal health

There were no major health problems with the piglets and only an occasional sign of scouring in the **LA** treatment, which did not require veterinary intervention. A summary of veterinary interventions is given in table 4.3.

Table 4.3 Summary of veterinary interventions and medications (Experiment 4).

Problem	*Pediococcus acidilactici* diet (PA)	Lactic acid diet (LA)	Intervention
Joint ill	2		1 ml injection of long acting Duphamox[a]
Aural haematoma		1	1 ml injection of Duphapen[b] given on 3 consecutive days
Physical ear injury		1	1 ml injection of long acting Duphamox

[a] Duphamox (Solvay Duphar Veterinary, Southampton)
[b] Duphapen (Solvay Duphar Veterinary, Southampton) active ingredient Procaine Penicillin/ Dihydrostreptomycin sulphate

4.8.2 Performance characteristics

The biological performance and effluent output of the pigs is summarised in table 4.4. Taken over the whole 28 days, treatment had no significant overall effect on any of the parameters of performance measured. The overall daily gain was 536 g d^{-1} and 563 g d^{-1} and the dry matter FCR 1.15 and 1.11 for the **LA** and **PA** treatments respectively. There were no significant treatment effects on water usage or effluent production.

Table 4.4 Performance of pigs fed liquid feed with the addition of an inoculant *Pediococcus acidilactici* (**PA**) or lactic acid (**LA**).

Parameter	Period	PA	LA	s.e.d.
Dry matter feed intake (g d^{-1})	week 1	277	296	44
	week 2	490	548	58
	week 3	630	645	97
	week 4	747	762	103
	Overall	536	563	71
Daily gain (g d^{-1})	week 1	299	366	42
	week 2	467	506	37
	week 3	559	568	43
	week 4	571	546	36
	Overall	474	496	25
Dry matter feed conversion ratio	week 1	0.99	0.77	0.15
	week 2	1.06	1.07	0.07
	week 3	1.13	1.14	0.06
	week 4	1.34	1.37	0.12
	Overall	1.15	1.11	0.09
Total water intake (ml pig d^{-1})	week 1	1090	1129	183
	week 2	1714	2237	258
	week 3	2484	2721	221
	week 4	3026	3047	391
	Overall	2078	2283	252
Average water intake from drinkers (ml pig d^{-1})	week 1	299	283	79
	week 2	312	670	132
	week 3	620	811	191
	week 4	814	790	221
	Overall	511	638	133
Average effluent production (ml pig d^{-1})	week 1	417	564	251
	week 2	911	1259	318
	week 3	1305	1680	244
	week 4	1837	1933	230
	Overall	1118	1359	185

4.8.3 Microbiology of the liquid feed system

A microbiological assessment of the raw diets as supplied indicated that Diet 1 contained an indigenous population which comprised very small numbers of coliform bacteria but had a small population of yeasts and lactic acid bacteria. Diet 2 contained larger numbers of indigenous coliform bacteria, similar numbers of yeasts and a larger number of lactic acid bacteria than Diet 1 (Table 4.5).

Table 4.5 Microbiological assessment of the indigenous populations of microorganisms present in the initial dry feed components of Diets 1 and 2.

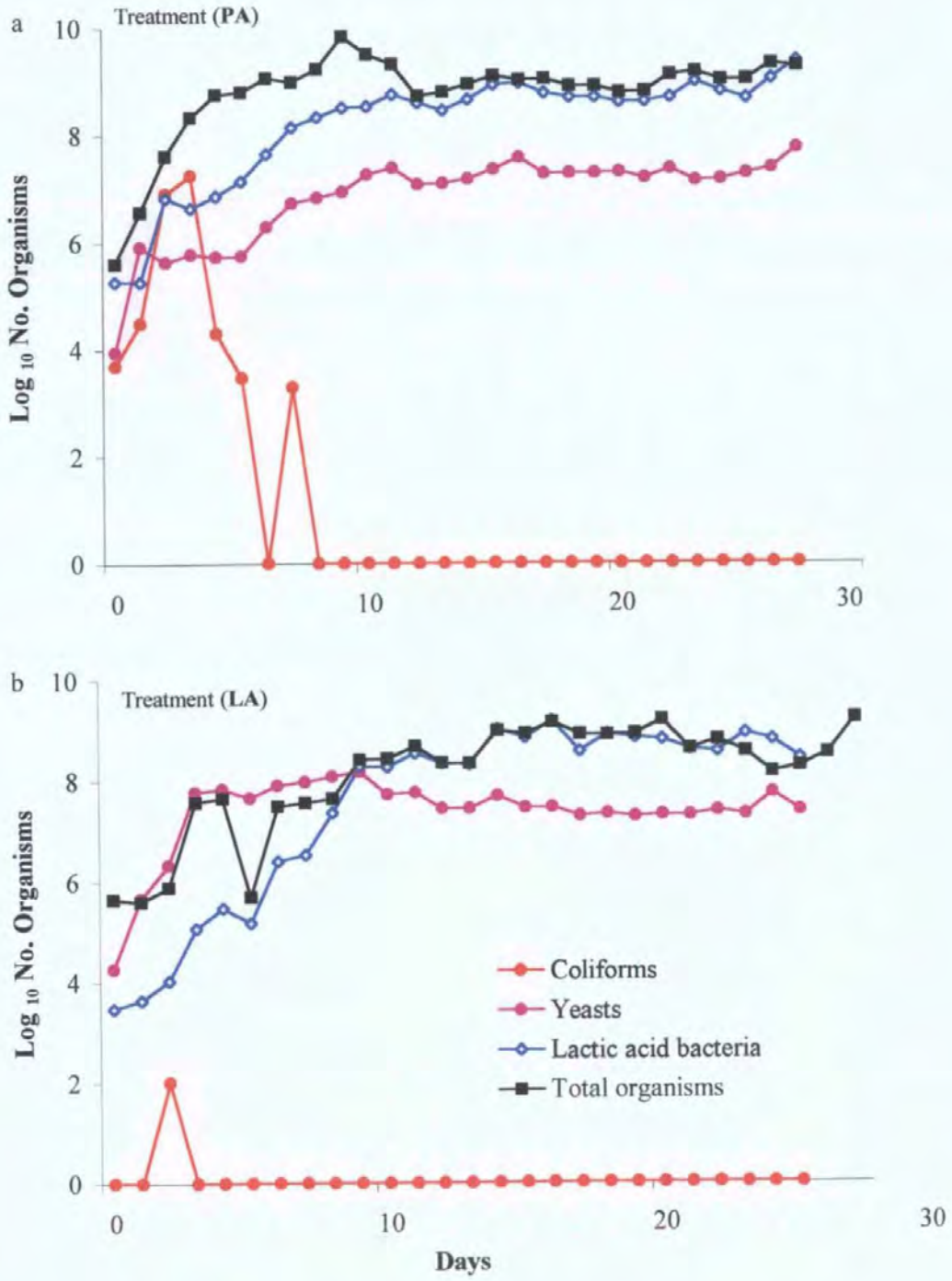
Diet	Day	Coliforms[a]	Yeasts[a]	Lactic acid bacteria[a]
1	1	0	3.3	3.63
1	7	1	3.32	3.21
2	14	2.84	3.69	4.17
2	21	2.84	3.32	4.05

[a] Log_{10} number of organisms

The microbiology of the liquid feed system was studied for both treatments. The changes in Log_{10} numbers of coliforms, lactic acid bacteria and yeasts with time are presented in figures 4.3 a,b.. For both treatments the numbers of yeasts increased rapidly over the first four days and thereafter remained relatively stable.

In treatment **PA** the numbers of lactic acid bacteria increased slowly from day 1 to day 10, thereafter remaining relatively stable. A coliform bloom was observed in the liquid feed in the first four days of the **PA** treatment and coliforms were not eliminated until day 10 of the experiment when the pH had been reduced to 4.5. In treatment **LA** a natural colonisation by lactic acid bacteria developed slowly in the liquid feed system (observed to be gram positive rods) reaching a peak on day 10 of the experiment and thereafter remaining relatively stable. In **LA** controlling the pH to 4.0 resulted in the elimination of coliforms after day 2.

Figure 4.3 The total microbiology in the liquid feed system with the addition of either lactic acid (LA) or *Pediococcus acidlilactici* (PA).



The relationship between pH and coliform numbers in both liquid feed systems is presented in figures 4.4 a,b.

As a result of yeast activity in both liquid feed systems alcohol was produced (Figure 4.5 a,b).

As the yeast populations increased rapidly in the first 9 days of the experiment the alcohol content of both the liquid feeds increased from 2.44 and 1.47 on day 1 to 4.66 and 3.81% w/v on day 9 for treatments **LA** and **PA** respectively. After day 9 the level of alcohol in both liquid feeds gradually decreased and stabilised at approximately 1.27 5 w/v (**LA**) and 1.44% w/v (**PA**) for the remaining 14 days of the experiments. The alcohol content did not appear to reduce the acceptability of the diet to the piglets. The alcohol content of the diet may have been responsible for changes in behaviour of the pigs, as the pigs on this study appeared more restful than pigs on previous studies.

4.8.4 Temperature

The temperature of the liquid feed system was recorded for both treatments and the relationship between total microbial activity and temperature is presented in figures 4.6 a,b. The temperature of the liquid diets increased sharply (by 5.3°C and 3.8°C for treatments **PA** and **LA** respectively) over the first five days. The temperature in PA remained relatively stable thereafter. However, in **LA** there was a sharp decline in temperature (6.6°C) from day 18 until day 22 before the temperature stabilised at approximately 15°C. This decline in temperature in the liquid feed system of treatment **LA**, coincided with a small decline in the Log_{10} numbers of total microorganisms.

Figure 4.4 The relationship between pH and coliform numbers in the liquid feeding system with the addition of either lactic acid (**LA**) or *Pediococcus acidilactici* (**PA**).

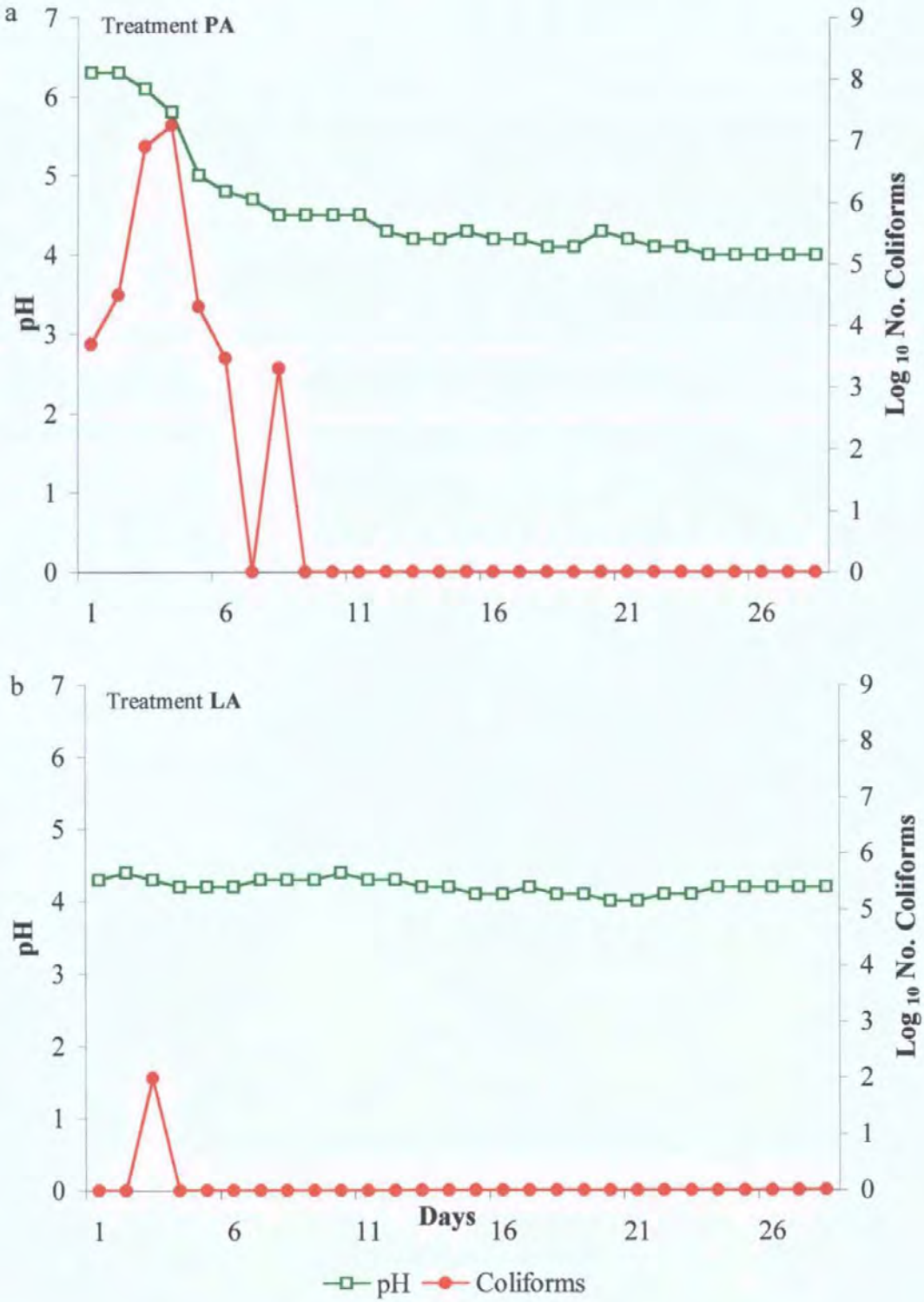


Figure 4.5 The relationship between the number of organisms and alcohol production in a liquid feed system with the addition of either *Pediococcus acidilactici* (PA) or lactic acid (LA).

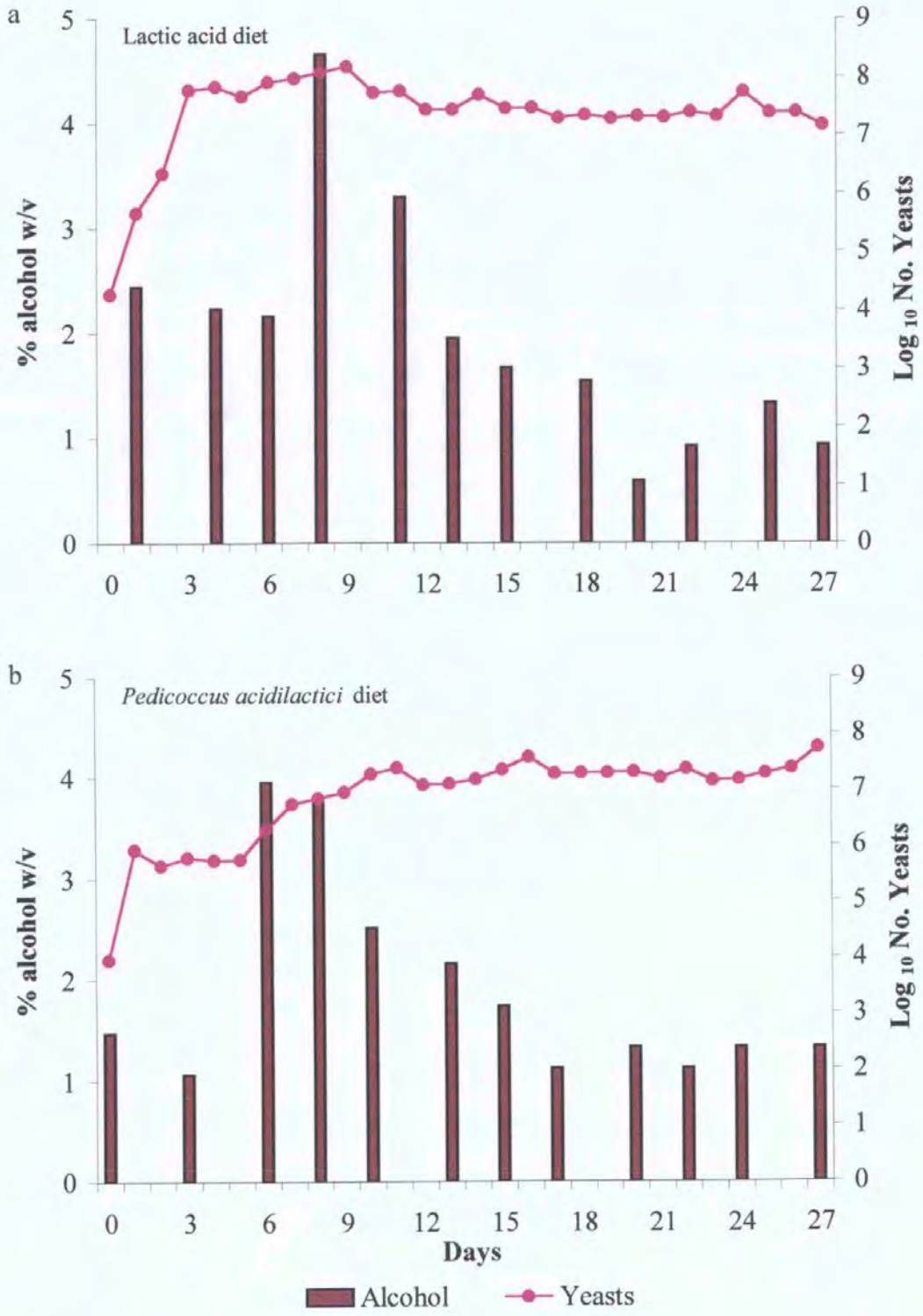
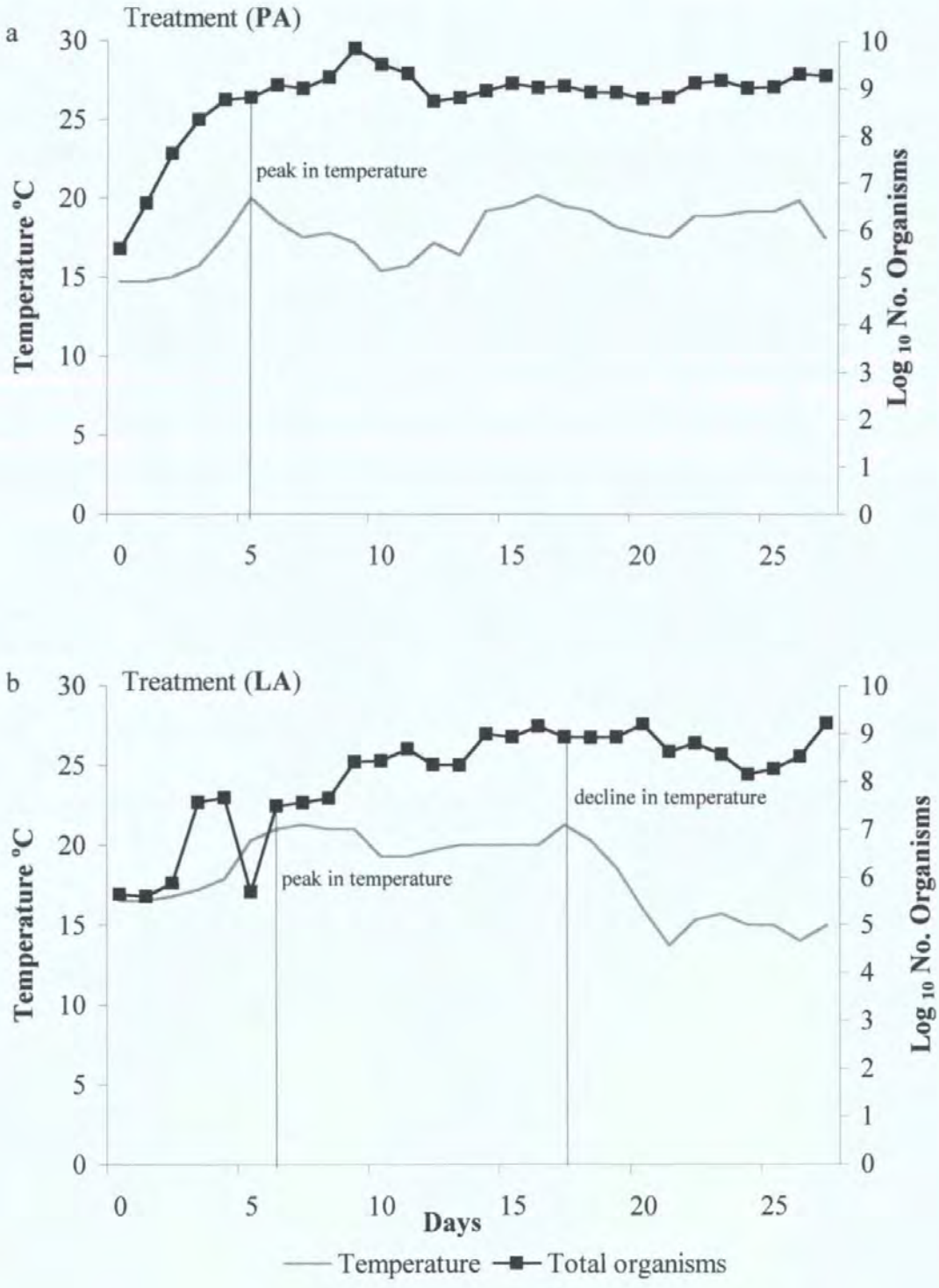


Figure 4.6 The relationship between microbial activity and temperature in the liquid feed system with the addition of either *Pediococcus acidilactici* (PA) or lactic acid (LA).



4.8.5 Changes in the concentrations of sugars

Changes in the concentrations of the sugars; glucose, sucrose, maltose and lactose were studied for both liquid feed systems until day 6 of the experiments when further readings were discontinued due to lack of definition in the recorded peak heights. The only noticeable change occurred in the level of glucose which decreased rapidly over time in treatment **PA** and more slowly in treatment **LA**.

4.8.6 Proximate analysis of the liquid feeds

The results of a proximate analysis, conducted at weekly intervals for both treatments is presented (Table 4.6).

Table 4.6 Proximate analysis of the dietary components, and calculated energy values of the liquid diets in Experiment 4, expressed as g kg^{-1} of dry matter.

Treatment	Week	Crude protein	Crude fat	Crude fibre	Ash	Nitrogen free extractive	Energy[c]
PA[a]	1	225.03	49.88	17.03	49.25	658.81	16.13
PA[a]	2	262.31	29.31	23.63	58.19	626.56	15.42
PA[a]	3	264.64	56.07	24.26	60.61	594.42	15.81
PA[a]	4	265.43	64.80	25.03	63.90	580.84	15.87
LA[b]	1	202.30	66.95	14.60	40.80	675.35	16.63
LA[b]	2	257.76	40.32	20.33	57.76	623.83	15.72
LA[b]	3	285.47	52.63	23.03	58.59	580.28	15.82
LA[b]	4	297.80	45.96	27.90	57.24	571.10	15.58

[a] Treatment *Pediococcus acidilactici*;
[b] Treatment Lactic acid;
[c] Megajoules (MJ) of digestible energy (DE) kg^{-1} of dry matter (DM).
Calculated using the equation ME (MJ kg^{-1} DM) = 0.016NFE + 0.032OIL + 0.018CP - 0.015CF Equation No. 8.22 (Whittemore 1993).
The ME was assumed to be 0.96% of DE.
Where ME = metabolisable energy, NFE = Nitrogen free extractive, OIL = Crude fat, CP = Crude protein, CF = Crude fibre.

The components of the liquid feed; crude protein, crude fat, crude fibre and ash changed

very little between treatments or with time, and were in similar proportions to those reported in table 4.1. Any observed changes in nitrogen free extractive for both treatments with time corresponded to the changing proportions of cereals and lactose in Diets 1 and 2. The average energy value of the dry matter of the liquid diets for both treatments was calculated as 15.80 MJ DE kg^{-1} DM for PA and 15.93 MJ DE kg^{-1} DM for LA throughout the feeding period.

4.9 Discussion and Conclusions

The results of this study demonstrated that treating the liquid feed with either lactic acid or *Pediococcus acidilactici* produced excellent results in terms of the biological performance of the weaner pigs, and that there was no significant difference between treatments. Dietary supplementation of weanling pig diets with organic acids has been used to improve growth performance (Falkowski and Aherne 1984; Geisting and Easter 1985; Fallon 1987; Risley, *et al.* 1991). In many experiments organic acids have been added to dry feed for pigs, and sometimes to their drinking water (Cole, Beal and Luscombe 1968a). Lactic acid, which has been used extensively to preserve human foods against spoilage organisms (Doores 1993), and has also been added to weaner diets. For example, Roth *et al.* (1993), supplemented the diet of weaner pigs 7 kg to 28 kg liveweight, fed *ad libitum* with different levels of lactic acid, and demonstrated that lactic acid improved the daily gain, feed intake and feed conversion ratio. They concluded that the most effective dosage of lactic acid added to a dry diet was 1.6%. In the current study the percentage of lactic acid added to a liquid diet was 1.25%. Fallon (1987) suggested that the greatest response to the use of organic acids will be obtained when piglets are weaned at 21 days or less. In this study the piglets were on average 24 ± 4 days which would place them just outside of the range Fallon (1987) has suggested as the optimum to benefit from acidification. There is no information available on the addition of lactic acid to liquid feed for weaner pigs. It

is known that newly weaned piglets have insufficient gastric secretions to optimise protein digestion (Kidder and Manners 1978) and that supplementation with organic acids can help to overcome this problem. The piglet would normally produce lactic acid in the stomach during suckling through the conversion of lactose in sow's milk by lactic acid bacteria. However, the abrupt process of weaning from liquid sow's milk to dry feed removes the piglet from this source of acidification and is further complicated by feeding newly weaned piglets diets which have a high buffering capacity. In this experiment the transition from sow's milk to a liquid feed which had been acidified by either lactic acid or fermented to produce lactic acid, removed two of the challenges that dry fed weaned piglet would have had to overcome. Namely, that the diet continues to be in a liquid form which it is used to and furthermore it is acidified to pH 4.0 which represents the level of the stomach pH of a piglet aged between 21 and 30 days. This may partly explain the reason for the excellent performance of pigs in this experiment.

It has been hypothesized that supplementation of the diet with organic acids reduces gastrointestinal pH and coliform numbers (Kirchgessner and Roth 1982; Burnell, Cromwell and Stahly 1988). Lactic acid has also been used as an effective means of controlling pathogenic organisms by administration in the drinking water of weaned piglets (Kershaw *et al.* 1966; Cole *et al.* 1968a). They demonstrated that the numbers of haemolytic *Escherichia coli* were reduced in the gastrointestinal tract of weaned piglets which had been given lactic acid in their drinking water compared to the control pigs. In this study it was demonstrated that although lactic acid was effective in controlling coliform bacteria in the liquid feed system, it was not a totally effective means of controlling natural fermentation, since a natural lactic acid fermentation still developed.

Although organic acids can be used to overcome some of the challenges of weaning young

piglets the use of lactic acid fermented liquid feed could be regarded as an alternative approach. In this experiment the lactic acid fermentation was controlled using *Pediococcus acidilactici.* As there was no difference in performance of the piglets between treatments, fermentation with **PA** would provide advantages over lactic acid as it is a cheaper option. It was calculated that the cost of using the inoculant *Pediococcus acidilactici* in treatment **PA** was £0.21 t^{-1} of dry matter feed, whereas the cost of adding lactic acid was calculated to be £148.89 t^{-1} dry matter. Providing that the correct inoculants are used in liquid feeding systems the potential for very large savings per tonne of feed exists compared with using organic acids.

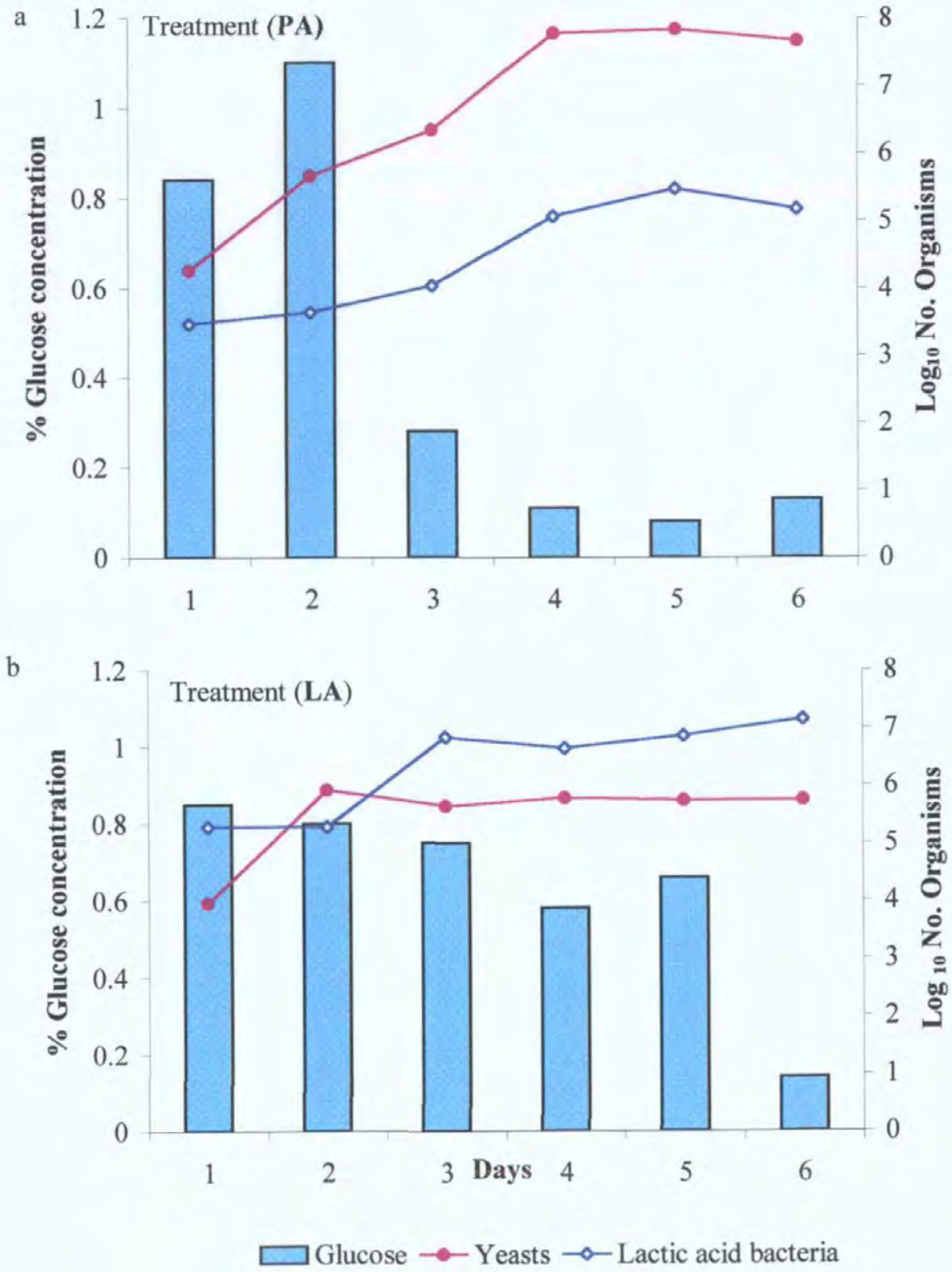
There are very few studies which have examined the effects of lactic acid fermented diets on pigs. However, *Lactobacillus* fermentation products have been shown to stimulate growth and improve feed efficiency in pigs (Hale and Newton 1979; Pollman *et al.* 1980) (Lessard and Brisson 1987) and feeding live Lactobacilli cells has been shown to reduce scouring in pigs (Muralidhara *et al.* 1977). The very limited published data available on the effects of lactic acid fermented diets on pigs is mainly concerned with its use in ensiled poultry or fish offal (Tibbetts, Seerley and McCampbell 1987; Urlings *et al.* 1993; Rose, Anderson and White 1994). Urlings *et al.* (1993) have suggested that the three main conditions for an optimal lactic acid fermentation are 1) addition of a sufficient amount of fermentable carbohydrates, 2) reduced oxygen during the fermentation process and storage of the fermented product, and 3) rapid multiplication of the starter culture and sufficient production of lactic acid.

In this study some of these conditions were met, for example extra glucose was included in the formulation of the diets to provide an easily fermentable carbohydrate substrate for the lactic acid bacteria starter culture *(Pediococcus acidilactici).* However, the *Pediococcus*

acidilactici organism used in this study did not reduce the pH of the diet to as low a value or as fast as the naturally occurring lactic acid bacteria spp. had done in previous experiments in this study, nor did **PA** become the dominant organism in the liquid feed system. This may have been because either the temperature or the pH in the liquid feed system was not at the optimum for the growth of *Pediococcus acidilactici* or, because a more aggressive strain of lactic acid bacteria was able to out compete it. In the current study the development of a population of yeasts (which would have produced alcohol) in both of the liquid feed systems did not appear to have any detrimental affect on the piglets. However, in liquid feeds where high sugar contents are present, there could be a problem of the alcohol content increasing to unacceptable levels which might affect the health of the piglets. In this system it might have been expected that a small proportion of dry matter would have been lost as a result of fermentation. However, the successful growth rates and feed efficiency of the piglets combined with their good health would have outweighed the costs of these losses. When starting up a liquid feed system glucose may assist in establishing rapid colonisation of feed by some species of lactic acid bacteria, but will also increase the rate of multiplication of any yeast species which may be present in the initial dry feed components of the diet (Table 4.5).

From the data in figures 4.7 a,b, it can clearly be seen that the rapid reduction in glucose substrate from the liquid feed in both systems coincides with a rapid increase in both yeasts and lactic acid bacteria. The utilisation of substrate, such as glucose, by yeasts or some species of lactic acid bacteria, raises a question, whether the energy content of the liquid diet be affected by the metabolism of these microorganisms to the detriment of the piglets which are to be fed on this diet. It is likely that energy is being lost from the liquid feed system as a result of heat, carbon dioxide and the multiplication of the organisms themselves.

Figure 4.7 The relationship between glucose and the activity of yeasts and lactic acid bacteria in the first six days in the liquid feed system with the addition of either *Pediococcus acidilactici* (PA) or lactic acid (LA).



When the energy contents of both of the liquid diets were calculated (Table 4.6) it was found that there appeared to be a loss of energy, especially between the first and second week of fermentation, (0.71 and 0.91 MJ DE kg^{-1} DM) for **PA** and **LA** respectively. However, it should be noted that when liquid diets are subjected to a proximate analysis any alcohol or volatile fatty acids present would be driven off as a volatile fraction and would not be accounted for in the energy value using the equation given by (Whittemore 1993). It was known that alcohol was present in both of the liquid diets in this study, therefore, this apparent decrease in energy value may be attributed to some extent to the alcohol fraction which was not accounted for by Whittemore's equation.

In this experiment an attempt was made to control the fermentation of a liquid diet for weanling pigs to provide a diet which would have a pH similar to suckling pigs and to provide a diet which would have the potential to reduce pathogenic bacteria thereby improving the biosecurity of the feed. Urlings (1993) concluded that feeding lactic acid fermented products to pigs can result in a reduction of the prevalence of certain enteropathogenic bacteria, such as *Salmonella* and *Escherichia coli.* It was demonstrated in this experiment that inoculating liquid diets with a lactic acid bacteria or acidifying with DL-lactic acid can be used as a successful technique to overcome some of the post weaning challenges which the newly weaned piglet faces.

4.10 POOLED DATA ON FEEDING TRIALS

Growth rate and feed efficiency of the pigs in Experiment 4 was superior to those in all previous Experiments in this study where liquid feed was fed (Table 4.7).

Table 4.7 Comparison of growth performance and total water intake for newly weaned piglets fed on liquid diets with a dry matter concentration of 255 g kg^{-1}.

	Experiment				
Parameter	1 (Trial 1)	1 (Trial 2)	2 (DM255)	4 (PA)	4 (LA)
Feed intake (meal equivalent intake g d^{-1})	807	654	475	536	563
Daily gain (g d^{-1}) week 1	123	178	187	277	296
Daily gain (g d^{-1}) overall	428	454	403	474	496
Dry matter feed conversion ratio	1.89	1.44	1.20	1.15	1.11
Total water intake (ml pig d^{-1})	2298	2028	1813	2078	2283

From the data in table 4.7 it would appear that dry matter feed intake (DMFI) and total water intake were higher in the first week post weaning in Experiment 4 than in Experiment 2. This increase in DMFI would have improved the physiological development of the piglets. As a consequence average daily liveweight gain of the pigs in Experiment 4 was superior both in the first week post weaning and overall compared with the pigs in Experiment 2. The post weaning growth check was greatly reduced when compared with similar pigs in Experiment 1. In Experiment 1 piglets on dry feed grew at only 62 g d^{-1} in the first week post weaning whereas the piglets in Experiment 4 grew at 277 g d^{-1} on PA and 296 g d^{-1} on LA.

When the final weights (28 days post weaning) and total gains of the piglets in Experiment

4 are compared to previous Experiments 1 and 2, it can be demonstrated that this particular Experiment (4) produced the highest overall total gain in weight (Table 4.8). The piglets in Experiment 4 were growing at a rate of 536 g d^{-1} and 563 g d^{-1} overall for **PA** and **LA** respectively which exceeds the rate of gain of the MLC top third rearing herds by 61 and 88 g d^{-1} for **PA** and **LA** respectively. Similarly feed conversion ratios in Experiment 4 were 1.15 and 1.11 which are considerably better than the result of MLC top third rearing herds (1.73).

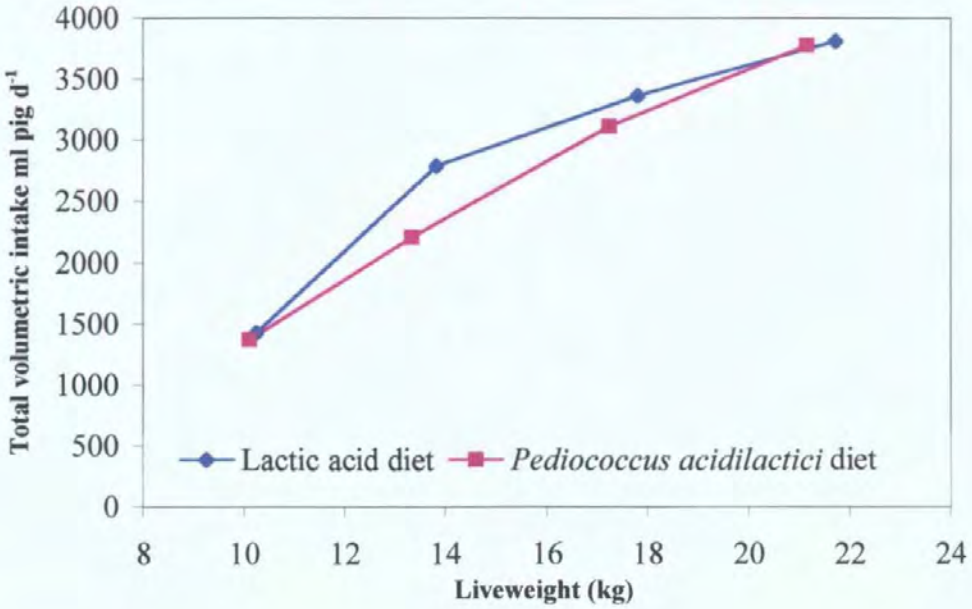
The results presented in table 4.7 clearly show that the overall total water intake was higher in Experiment 4 (2078 and 2283 ml pig d^{-1}, **PA** and **LA** respectively), than for those pigs fed an equivalent dry matter concentration in Experiment 2 (1813 ml pig d^{-1}). The total volumetric fill for the pigs in Experiment 4 were calculated to be 18% of the liveweight for pigs for both treatments **PA** and **LA** (Figure 4.8). On treatment **DM255** and **DM224** (Experiment 2) total volumetric fill was 19%, therefore, there was a surprisingly good consistency between Experiments 2 and Experiment 4.

Piglets still had a requirement for water as water, which was equivalent to 575 ml d^{-1} overall in Experiment 4. This exceeded the requirement for water of 423 ml d^{-1} overall for pigs fed a similar diet with the same dry matter concentration in Experiment 2. The extra water consumption was not considered to be due to an imbalance of minerals because the diets were very similar in composition between Experiments 2 and 4. Rather it was considered to be as a consequence of the increased DM feed intake which would have required a greater intake of water for metabolism.

Table 4.8 Comparison of weaning weights, final weights and total gains of newly weaned piglets.

Experiments	1	1	2	2	2	2	4	4
Diets	Trial 2 (dry fed)	Trial 2 (liquid fed)	Dry matter of 255 g kg^{-1}	Dry matter of 224 g kg^{-1}	Dry matter of 179 g kg^{-1}	Dry matter of 149 g kg^{-1}	*Pediococcus acidlactici*	Lactic acid
Parameter								
Weaning weight (kg)	6.8	6.7	7.0	7.3	7.5	7.0	8.0	7.7
Final weight (kg)	18.0	19.4	18.3	17.0	17.8	17.6	21.3	21.6
Total Gain (kg)	12.7	11.1	11.3	9.6	10.2	10.6	13.3	14.0

Figure 4.8 The relationship between total volumetric intake and liveweight of piglets fed on a diet with the addition of either *Pediococcus acidilactici* (PA) or lactic acid (LA).



4.10.1 Pooled data set 1

Data was pooled from Experiments 1 (Trial 2), 2 and 4, to provide a data set (n = 224 observations) for the analysis of factors affecting growth rates of newly weaned pigs. The mean weaning age of the piglets was 23 ± 3 days, and the mean weaning weight of the piglets was 7.15 ± 1.92 kg. Linear regression was used to establish the relationship between weaning age (WA) and weight 28 days post weaning (PWW). The relationship was described by Equation 4.1.

$$28 \text{ day PWW} = 11.8 + 0.300\text{WA} \quad \text{R-sq (adj)} = 10.9\% \qquad \text{(Equation 4.1)}$$

Weaning age had a significant ($P < 0.001$) effect on 28 day PWW. Linear regression was also used to establish the relationship between weaning weight (WW) and 28 day PWW of the piglets. The relationship obtained is described by Equation 4.2.

$$28 \text{ day PWW} = 7.74 + 1.56\text{WW} \quad \text{R-sq (adj)} = 25.6\% \qquad \text{(Equation 4. 2)}$$

The pooled data revealed that weaning weight had a significant ($P<0.001$) effect on weight 28 days post weaning.

When weaning weight was regressed against weaning age there was a linear relationship between them ($P<0.01$) which implies that they are not totally independent of each other. Therefore multiple regression analyses were undertaken including either weaning age or weaning weight as a factor in order to separate the effects of these parameters (Equation 4.3 and 4.4).

$$28 \text{ day PWW} = 9.51 + 0.271\text{WA} + 0.473\text{T} + 0.482\text{S} \quad \text{R sq (adj)} = 23.3\%$$

(Equation 4.3)

28 day PWW = 6.30 + 1.41WW + 0.419T + 0.332S R sq (adj) = 34.9%

Where (T) = treatment; (S) = sex; (Equation 4.4)

It might have been expected that weaning age would have an affect on final 28 day weight since newly weaned piglets are physiologically immature and often experience growth check in the first week post weaning.

Multiple regression analysis was used to establish the effect of treatment, sex, weaning weight and weaning age on 28 day post weaning weight and to apportion the components of variation (Equation 4.5).

28 day PWW = 2.17 + 1.30WW + 0.399T + 0.215 WA + 0.306S

R-sq (adj) = 40.3% (Equation 4.5)

Weaning weight, weaning age and treatment each had a significant ($P<0.001$) effect on 28 day PWW. When the variation in the data was apportioned, weaning weight accounted for 25.9%, treatment 9.5% and weaning age 5.7% of the variation while sex accounted for only 0.3%. Even though weaning weight and weaning age are not independent of each other the inclusion of both factors explains more of the variation (Equation 4.3).

4.10.2 Pooled data set 2

From the review of the literature it is apparent that nutrient intake in the first week post weaning may have a significant effect on 28 day final weights. Since it is known that liquid feeding encourages nutrient intake only the liquid fed pigs were used to construct a second data set (n = 186). The mean weaning weight of the piglets in this data set was 7.23 ± 0.96 kg and the weaning age was 24 ± 3.07 days. Multiple regression was used to

examine the relationship between dry matter food intake (DMFI) in the first week post weaning (0 - 7 days) and 28 day PWW. The relationship is given in Equation 4.6.

28 day PWW = 15.0 + 2.49 DMFI kg^{-1} x 0 - 7 days post weaning DMFI kg^{-1}

R-sq (adj) = 39.9% (Equation 4.6)

The data indicated that DMFI of newly weaned piglets in the first week post weaning, had a highly significant ($P<0.001$) effect on 28 day PWW. Equation 4.6 could be used to predict the effect of increasing food intake in the first week post weaning on the 28 day PWW of piglets. For example, for a piglet eating 75 g d^{-1} in the first week post weaning (the worst feed intake recorded in Experiment 4) the calculation for 28 day PWW will be as shown in Equation 4.7.

28 day PWW = 15.0 + 2.49 x 0.53 kg = 16.32 kg (Equation 4.7)

When the value for a piglet eating 433 g d^{-1} (the best feed intake recorded in Experiment 4) are substituted in the equation the calculation is given in Equation 4.8.

28 day PWW = 14.0 + 2.49 x 3.03 kg = 22.54 kg (Equation 4.8)

Within the range tested increasing food intake by 10 g d^{-1} gave an increase in 174 g in weight 28 days later.

4.10.3 Summary on pooled data

In summary the pooled data demonstrated that both weaning age and weaning weight had a significant effect ($P<0.001$ and $P<0.001$ respectively), on 28 day PWW, and that weaning

weight and weaning age are not independent variables. Dry matter food intake in the first week post weaning had a highly significant ($P<0.001$) effect on the final 28 day weight.

CHAPTER 5 IMPROVING THE USE OF FOOD INDUSTRY LIQUID RESIDUES BY FERMENTATION

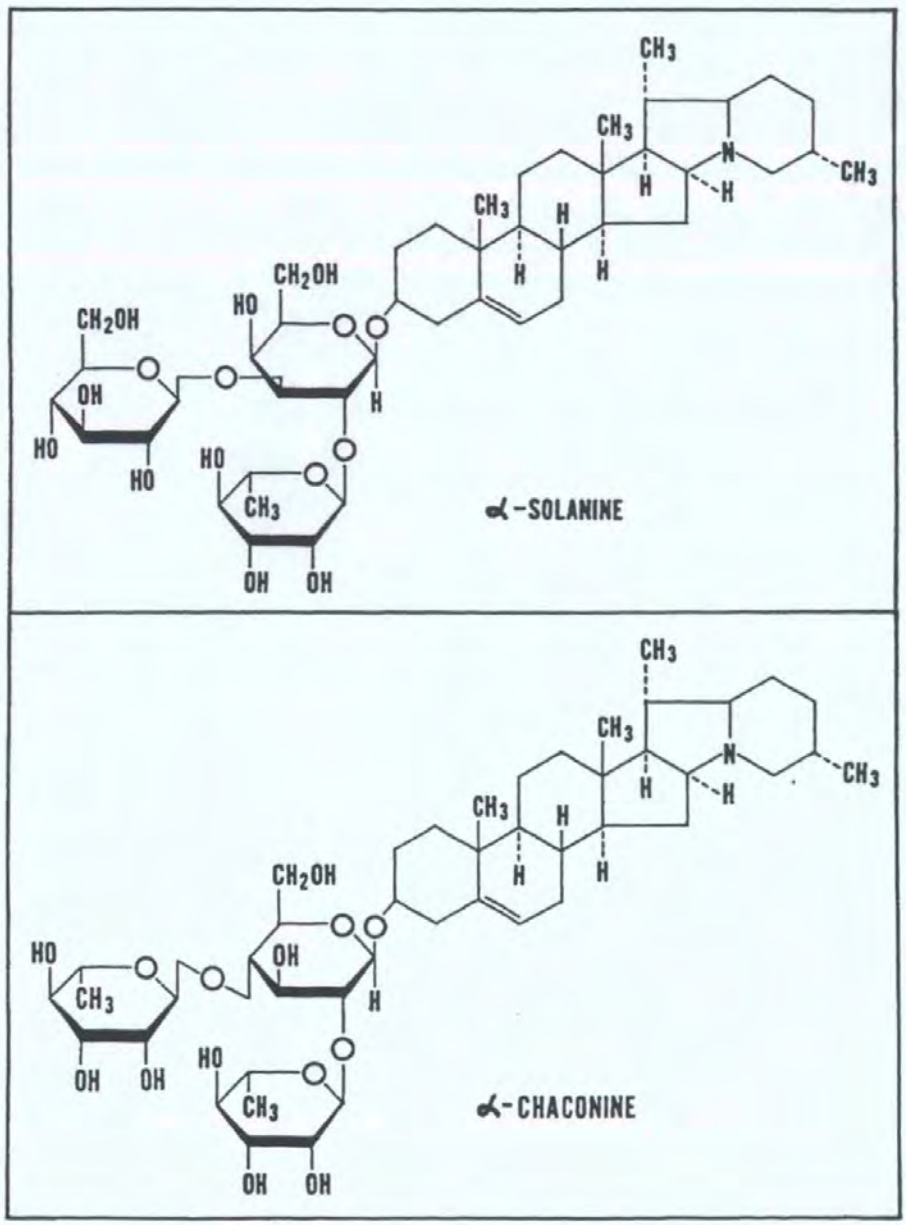
EXPERIMENT 5 STEEPING AS A MEANS OF IMPROVING THE USE OF REJECT RAW POTATOES FOR USE IN LIQUID FEED SYSTEMS FOR PIGS.

5.1 Introduction

The cultivated potato (*Solanum tuberosum*) is one of the world's major agricultural crops, consumed daily by millions of people (Maga 1980; Smith, Roddick adn Leighton-Jones 1996). The potato industry in the UK is based largely on home production of around 6 million t y^{-1}. Of this 845,000 t are either used as stock feed or are rejected (Ritson and Taylor 1991). The reject raw potatoes could be a useful source of nutrients for inclusion in liquid diets for pigs. However, reject raw potatoes which are unfit for human or stock feed consumption are most likely to have suffered from greening, physical damage, poor storage, excesses of heat, cold, light all of which contribute to the accumulation of toxic glycoalkaloids which are naturally present in the potato tuber (Maga 1980; Friedman and Dao 1992; Houben and Brunt 1994). Potatoes with obvious signs of greening can be expected to contain levels of total glycoalkaloids of 81.80 mg kg^{-1} fresh weight tuber (Moira variety) (De Maine, Bain and Joyce 1988). This will limit their use as a raw material for liquid pig diets. Producers who feed their pigs presently limit their use of reject raw potatoes in pig diets due to unpalatability and the high levels of mortality which occur (P. McTiffin personal communication 1996). The unpalatability of raw potato may be due to the glycoalkaloids present in the potato tuber which are known to impart a bitter taste (Bushway, Bureau and King 1986; Smith *et al.* 1996). Glycoalkaloids are toxic, naturally occurring compounds found in plants that are members of the Solanaceae family (Morris and Lee 1984; Carman, Kuan, Ware, Francis and Kirschenheuter 1986). The two major glycoalkaloids in commercial potatoes are α-solanine and α-chaconine (Friedman and

Dao 1992). The general structure of the glycoalkaloids α-solanine and α-chaconine present in potato tubers are presented in figure 5.1.

Figure 5.1 Chemical structure of the glycoalkaloids α-solanine and α-chaconine.



Glycoalkaloids are found throughout the potato plant with levels varying considerably among the different parts (Table 5.1).

Table 5.1 Levels of glycoalkaloids in various parts of the potato plant

Plant part	Glycoalkaloid concentration (mg kg^{-1} fresh weight)
Flowers	2150 - 5000
Leaves	230 - 1000
Stems	23 - 33
Roots	180 - 400
Bitter-tasting tuber	250 - 800
Whole tuber	10 - 150
Skin	300 - 640
Peel	150 - 1068
Flesh	12 - 100
Sprouts	2000 - 7300

After (Smith *et al.* 1996)

The toxicity of potato glycoalkaloids to humans has been reported as 3 - 6 mg kg^{-1} of body (Morris and Lee 1984; Houben and Brunt 1994). In the US the generally accepted safe upper limit of total glycoalkaloids (TGA) in potato tubers is 200 mg kg^{-1} (Carman *et al.* 1986). A recent review by Smith *et al.* (1996) reported that glycoalkaloid poisoning from potatoes is both high and variable, and that oral doses in the range of 1 - 5 mg kg^{-1} of body mass can be marginally to severely toxic to humans. It has been suggested that a new recommended safety level of 60 - 70 mg kg^{-1} of glycoalkaloids per 100 g tuber should be considered (Smith *et al.* 1996). There is a very narrow margin between safe levels of these glycoalkaloids, and the level which produces toxicity in humans. This could apply equally to pigs who possess a similar physiology to humans. It has also been demonstrated that boiling, baking or microwaving does not reduce the levels of glycoalkaloids in potatoes (Bushway and Ponnampalam 1981). No value has been found in the literature for safe

levels of glycoalkaloids of potatoes for inclusion in the diets for pigs.

Glycoalkaloids consist of a C_{27} steroidal alkaloid skeleton (aglycone) to which one or more sugar groups are attached. It is generally considered that the major glycoalkaloids in potatoes are α-solanine and α-chaconine (Maga 1980). They form about 95% of the total glycoalkaloids present in potatoes (Houben and Brunt 1994). If the increased levels of potentially toxic glycoalkaloids α-solanine and α-chaconine could be reduced to safe levels, then the remaining raw potato mash could be used to feed to pigs in a liquid diet.

Both α-solanine and α-chaconine contain similar groups of sugars attached to the aglycone backbone. The structure of these compounds suggests that steeping would cause disassociation in water. If this were the case α-solanine and α-chaconine could then be removed in the resultant liquor and the remaining raw potato mash could be safely fed to pigs.

Methodologies for analysis of glycoalkaloids have been extensively studied but no single method has gained widespread acceptance (Friedman and Dao 1992). However, high performance liquid chromatography (HPLC) is probably the method of choice (Houben and Brunt 1994). This method consists of three stages: 1) extraction of the glycoalkaloids with an aqueous solvent; 2) removal of interfering substance (impurities); and 3) analysis by HPLC.

The objective of this experiment was to assess the affects of steeping raw reject potatoes on the concentrations of α-solanine and α-chaconine.

5.2 Materials and Methods

5.2.1 Experimental design and treatment

Reject raw potatoes were chopped and steeped in water for 7 days at room temperature. A sample of the chopped potato was removed before and after soaking and the concentrations of α-solanine and α-chaconine were determined by HPLC analysis. The procedure was repeated twice.

5.2.2 Materials

α-solanine (purity >95%) and α-chaconine (purity >95%) and 1-heptane-sulfonic acid (purity >95%) were purchased from Sigma Chemical Co. (Dorset). All other solvents and reagents were of Analar grade (BDH Chemicals Ltd, Dorset) except for acetonitrile which was HPLC grade (Hipersolve™; BDH Chemicals Ltd, Dorset). Distilled water was used for the preparation of the extracting solution and the buffer. Fresh potatoes were purchased from local supermarkets.

5.2.3 Apparatus

The HPLC was performed using a Varian 9050 variable wavelength UV-VIS detector, fitted with a 20 µl injection loop. The UV detector operating at 202 nm, and data recorded on a Hewlett Packard HP 3395 integrator. A Nucleosil 5 C_{18} - AB (5 µl) column (250 x 4 mm ID) supplied by (Phenomenex UK Ltd., Macclesfield, Cheshire) was used to separate the glycoalkaloids at a pump-flow rate of 1 ml/min.

5.2.4 Solutions

The extracting solution was prepared by diluting 2 g of 1-heptanesulfonic acid with distilled water to 500 ml and 5 ml of concentrated acetic acid. The mobile phase was prepared by mixing acetonitrile with water (60:40, v/v). The mobile phase was degassed before use.

Glycoalkaloid standard solutions were prepared by dissolving α-solanine (0.50 mg ml^{-1}) and α-chaconine (0.50 mg ml^{-1}) in 2 ml of acetonitrile-buffer (60:40 v/v). The stock solutions were stored at 4°C in a refrigerator.

5.2.5 Procedures

Standards were run three times until the concentrations of α-solanine and α-chaconine could be determined with an accuracy of > 0.99 %.

The techniques of sample preparation were practised several times before the main experiments began in order to reduce variation. Fresh potato samples consisted of approximately 2 kg of fresh, whole reject raw potatoes (6 to 10 potatoes) of mixed varieties, discarded from the local supermarket and showing signs of bruising, sprouting and greening. From this sample approximately 1 kg were washed, wiped dry and chopped into (*circa* 2 cm) pieces and placed in a 2 l beaker which was filled with water to give a total volume of 2 l. A 200 g sample of the chopped potato was removed immediately for analysis. The remainder was left to soak at room temperature for 7 days. The pH of the liquor was measured each day. The initial 200 g sample was macerated to a smooth consistency in a food processor. Sodium bisulfite was added at the rate of 1g sodium bisulfite to a 100 g of sample to retard oxidation during sample preparation and analysis.

A 100 g portion of the prepared sample was removed for analysis and placed in a food processor with 120 ml of the extracting solution. The sample was blended at high speed for 3 min and filtered through coarse paper, with vacuum suction, to obtain a crude filtrate. For weight/volume calculations, all samples were assumed to have a water content of 80% (Carman *et al.* 1986). This resulted in a sample to solvent ratio of 100g of sample to 200 ml of solvent.

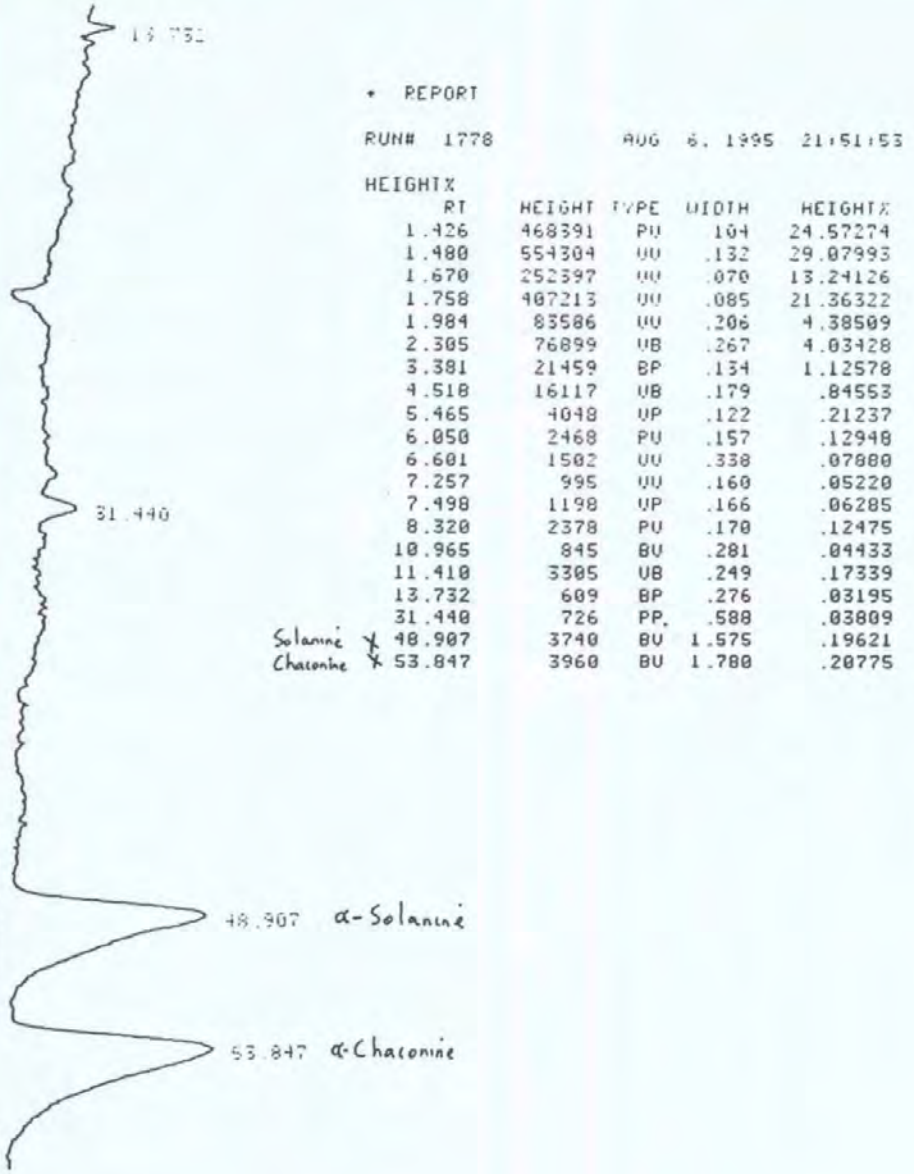
Glycoalkaloids from the potato samples were concentrated using solid-phase extraction (SPE) with a disposable Sep-Pak C_{18} (Millipore Waters, Harrow, Middlesex). The glycoalkaloids were extracted from the potato sample by ion-pair extraction using the extracting solution. After thoroughly mixing the sample, insoluble constituents were removed by filtration. The pre-treatment of the SPE column consisted of flushing with 5 ml of methanol, followed by 5 ml of the extracting solution. To the conditioned column was added 10 ml of the sample extract; it was allowed to pass through the column, and then was followed by 5 ml of acetonitrile-water (20:80 v/v) to remove any interfering constituents of the sample. All of the previous elutes were discarded. Finally, the glycoalkaloids were eluted from the Sep-Pak C_{18} column with two 1 ml volumes of acetonitrile-buffer (60:40 v/v).

In this experiment the levels of α-solanine and α-chaconine were measured using the methods of (Carman *et al.* 1986) and (Houben and Brunt 1994) with some minor modifications. HPLC was performed by injecting 20 µl of the eluted glycoalkaloids onto the liquid chromatograph. All HPLC samples were carried out in duplicate.

A recovery test was performed to verify how much of the glycoalkaloids were being eluted with acetonitrile buffer (60:40, v/v). To do this, standard solutions of (0.5 mg ml^{-1}) of both α-solanine and α-chaconine were treated in the same way as the method described for SPE and HPLC and compared to the same standard solutions which had not been passed through the Sep-Pak C_{18} column.

5.3 Results

As can be seen from the data presented in figure 5.2, the glycoalkaloids can be efficiently separated from other constituents by flushing the SPE column with acetonitrile-water (20/80, v/v).



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	4.518	16117	VB	.179	.84553
	5.465	4048	VP	.122	.21237
	6.050	2468	PV	.157	.12948
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	7.257	995	VV	.160	.05220
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	8.320	2378	PV	.170	.12475
	10.965	845	BV	.281	.04433
	11.410	3305	VB	.249	.17339
	13.732	609	BP	.276	.03195
	31.440	726	PP	.588	.03809
Solanine	48.907	3740	BV	1.575	.19621
Chaconine	53.847	3960	BV	1.780	.20775

* REPORT

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HEIGHT%

	RT	HEIGHT	TYPE	WIDTH	HEIGHT%
	1.471	281723	PU	.208	25.15682
	1.663	157589	VV	.059	14.07216
	1.735	494144	VV	.102	44.12520
	2.015	56917	VV	.214	5.08247
	2.349	43748	VV	.259	3.90653
	2.744	18446	VV	.249	1.64716
	2.998	11116	VV	.152	.99262
	3.242	7911	VB	.165	.70642
	3.755	3061	BP	.101	.27334
	4.335	6828	PV	.167	.60971
	5.120	856	VP	.104	.07644
	5.405	9491	PB	.108	.84751
	5.962	2899	BV	.108	.25887
	6.103	5781	VP	.116	.51622
	6.642	2566	PV	.290	.22913
	7.096	1831	VV	.184	.16350
	8.171	5153	PV	.150	.46014
	11.094	2040	BB	.198	.18216
	13.432	1456	PV	.275	.13002
	30.353	535	BV	.310	.04777
	49.612	1772	VV	.290	.15823
Solanine	49.970	2205	VV	1.062	.19690
Chaconine	55.077	1800	BV	.657	.16073

Figure 5.2 Separation of α-solanine and α-chaconine by high performance liquid chromatography.

The difference in the two sets of samples represented the recovery rate. The recovery rates were found to be 61.43% and 51.56% for α-solanine and α-chaconine respectively. The recovery rates were used to calculate the amounts of glycoalkaloids present in the samples before and after treatment. The results from the HPLC analysis of the samples are presented in table 5.2.

Table 5.2 Concentrations of α-solanine and α-chaconine in raw reject potatoes expressed as mg kg^{-1} of fresh weight raw potato.

Glycoalkaloid	Before steeping	After steeping	Reduction
α-solanine[a]	47.9	31.3	16.6
α-chaconine[a]	56.7	28.0	28.7
Total	104.6	59.3	45.3

[a] Concentration in mg kg^{-1} fresh weight raw potato, mean of two values

The data presented in table 5.2 demonstrate that steeping reject raw potatoes in water, at room temperature, reduced the levels of α-solanine and α-chaconine by 16.6 and 28.7 mg/100g respectively.

It was observed in this experiment that a natural fermentation of the steeped potato had occurred. This resulted in a reduction in pH over time from 6.0 to 4.6 by day 7.

5.4 Discussion and Conclusions

The results obtained in this study suggest that steeping could be used as a method for reducing the concentrations of α-solanine and α-chaconine in reject raw potatoes. However, the analytical method used in this experiment produced poor recovery rates, and could be improved by increasing the speed and duration of liquidising of the sample in a

commercial food processor. In the methods of Carman *et al.* (1986) and Houben and Brunt (1994) a Waring commercial food processor was used. This may have been more effective at extracting glycoalkaloids than the food processor used in this experiment. The initial concentrations of α-solanine and α-chaconine combined were 104.60 mg kg^{-1} of raw potato, which is 95% of the total glycoalkaloids. Although this is below the safety level set by the US at 200 mg kg^{-1} it is above the new recommended safety level of 60 - 70 mg kg^{-1} suggested by (Smith *et al.* 1996).

Assuming that the lethal dose is the same for pigs as for humans (3 - 6 mg kg^{-1} body weight), it can be calculated what this means in terms of feeding reject potatoes to pigs. For a finisher pig of 70 kg the minimum lethal dose (LD) would be 3 x 70 kg = 210 mg (TGA). This is equivalent to 1.05 kg of raw potato in one feed containing 200 mg kg^{-1} total glycoalkaloids (TGA) (US safety limit). The percentage of potato which can be included in a grower/finisher pig diet can replace 25% of the cereals (on a dry matter basis) (MAFF 1986). For a finisher pig aged 25 weeks MAFF (1984) recommend feeding 2.65 kg of food per day (total diet) of which approximately 50 % will be cereals. Assuming cereals have 87% dry matter and potatoes have 23 % dry matter (MAFF 1986) then the amount of potato which can substitute 25% of cereals will be 1.25 kg of fresh potato. If the pig eats 1.25 kg of potatoes twice daily it will receive the equivalent of 124 mg TGA in one feed. If the US safety level of 200 mg kg^{-1} TGA is applied, then the pig will not have received a lethal dose. However, if Smith's safety level is applied (60 - 70 mg kg^{-1}) then the pig will have consumed approximately twice as much TGA as Smith has recommended. The results of this study clearly demonstrate that steeping reduced the TGA levels to 69.3 mg kg^{-1} of raw potato which is within Smith's new recommended safety level.

EXPERIMENT 6. IMPROVING THE USE OF POTATO STEAMED PEELINGS (PSP) BY FERMENTATION.

5.5 Introduction

In the Netherlands it is estimated that 350,000 t of potato steamed peelings (PSP), which are liquid food industry residues, are currently being fed to pigs in liquid feeding systems, previously presented in table 1.3 (Chapter 1). Pig producers in the UK are also feeding PSP though no estimate of the quantity they use is available. However, although PSP is a good source of energy for the pig there are problems associated with its use. These problems have been identified as uncontrolled fermentation, high ash content, unpalatability and the production of sticky black faeces by the pigs fed on this raw material (Brooks and McGill 1995). The high viscosity of PSP, which is due to partly solubilised pectin and starch, can create additional problems with pumping in a liquid feed system. Amylase enzymes are currently added to reduce the viscosity of PSP (P. McTiffin personal communication 1996).

The alternative approach to the treatment of PSP is to used controlled fermentation with lactic acid bacteria. There is very little information available on fermented cooked potato products. However, one product produced in the Andes in South America is prepared from cooked potatoes which are allowed to ferment as they sun dry for 10 -16 days. The product "papa seca" has an active fermentation (produced by unidentified microorganisms) and is used for human consumption (Campbell-Platt 1987). Fermentation of PSP with lactic acid bacteria could have the effect of lowering the pH, thereby providing biosecurity, and hydrolysing the starch into simpler sugars. Any hydrolysis of the starch would be expected to reduce its viscosity and increase digestibility and palatability by the release of simple sugars.

The objectives of the experiment reported here were:

- to determine the optimum addition rate of *Pediococcus acidilactici* (PA) for use in PSP
- to compare changes in the pH viscosity and microflora of PSP with or without the addition of an inoculant

5.6 Materials and Methods

5.6.1 Experimental design and treatments

The experiment was conducted in two parts. The first part of the experiment consisted of constructing a dose response curve to determine the optimum levels of inoculant required to produce a rapid reduction in pH over 4 days. In the second part of the experiment a microbial assessment was conducted on the PSP during fermentation over 5 days. The materials, apparatus and sample preparation were the same for both treatments Part 1 and Part 2. Both treatments were duplicated. The treatments were as follows:

Treatments Part 1

T0	Control; PSP no inoculant
T1	PSP inoculated with 0.5 g PA kg^{-1}
T2	PSP inoculated with 0.05 g PA kg^{-1}
T3	PSP inoculated with 0.005 g PA kg^{-1}
T4	PSP inoculated with 0.0005 g PA kg^{-1}

Treatments Part 2

PSPN	Control; PSP no inoculant
PSPPA	PSP inoculated with 0.005 g PA kg^{-1}

5.6.2 Materials

Fresh potatoes were purchased from a local supermarket. The lactic acid bacterium used, *Pediococcus acidilactici* (Park Tonks Ltd., Cambridge, UK), supplied in freeze dried form and stored at 4°C in a refrigerator before reconstitution.

5.6.3 Apparatus

Viscosity was measured in treatments **T0**, **T1**, **T2**, **T3** and **T4**, using a rotating cylinder viscometer (Bohlin Visco 88). The food processor was a Moulinex Masterchef 500. pH was measured using a pH meter (Kent EIL 7015).

5.6.4 Sample preparation

This preparation emulates a close as is possible in a laboratory the process used to produce PSP (D. Borthwick personal communication 1995, Bird's Eye Manufacturers, Lowestoft, E.Anglia). PSP were prepared by peeling potatoes in water (approximately 3 kg of potato peel in total) and boiling the peel of the potatoes in sufficient water to cover them for 10 minutes. The peel and water were allowed to cool for 20 minutes. These were then added to a food processor and macerated at high speed, for 15 seconds, to produce a coarse mixture of potato peel and water.

5.6.5 Dose Response Curve

A 250 ml of prepared sample of PSP was added to five 400 ml beakers and inoculated at the rate of 0.5, 0.05, 0.005 and 0.0005 g PA kg^{-1} (**T1**, **T2**, **T3** and **T4**), or un-inoculated (**T0**). The beakers were covered with tin foil (to prevent cross contamination) and allowed to stand for 4 days at ambient temperature. The pH, temperature and viscosity of each sample was measured daily.

5.6.6 Microbiological Assessment

250 ml of the prepared sample of PSP was added to two 400 ml beakers One beaker acted as the control (**PSPN**) the second was inoculated with 0.005 g PA kg^{-1} (**PSPPA**). The beakers were covered with tin foil (to prevent cross contamination) and incubated aerobically at 37°C. The pH and temperature of each sample was measured daily. A microbiological assessment was made of each sample for lactic acid bacteria, total organisms and coliform bacteria using the methods described in Chapter 2, 2.2.1.

5.7 Results

5.7.1 Treatments Part 1

There was a significant difference ($P<0.001$) in pH between treatments as a result of inoculation, and over time ($P<0.001$) (Table 5.3). A more rapid reduction in pH *circa* 6.6 to 4.2 occurred in inoculated PSP with *Pediococcus acidilactici* than in the control which was allowed to ferment naturally *circa* 6.6 to 4.7. The optimum addition rate of PA needed to achieve this rapid rate of reduction in pH was 0.05 g PA kg^{-1} PSP treatment **T2** (Figure 5.3).

There was a significant difference ($P<0.001$) in viscosity between treatments as a result of inoculation, and over time ($P<0.001$) (Table 5.3). The viscosity of the control treatment (**T0**) was significantly less ($P<0.001$), than any of the inoculated treatments (Figure 5.4).

Table 5.3 Comparison of the mean effects of inoculation with *Pediococcus acidilactici* on potato steamed peelings on pH and viscosity.

	Inoculation rate g kg^{-1}					
Parameter	None	0.5g	0.05g	0.005g	0.0005g	s.e.d.
pH	5.77^{abcd}	5.28^{c}	5.24^{a}	5.26^{b}	5.40^{d}	.14 **
Viscosity	5.70^{abce}	7.42^{c}	6.86^{ef}	7.92^{bf}	8.20^{ad}	.44 ***

Means with the same superscript in the same row differ significantly ($P<0.05$).
* $P<0.05$; ** $P<0.01$; ***$P<0.001$.

Figure 5.3 The effect of graded additions of *Pediococcus acidilactici* (PA) on the pH of potato steamed peelings.

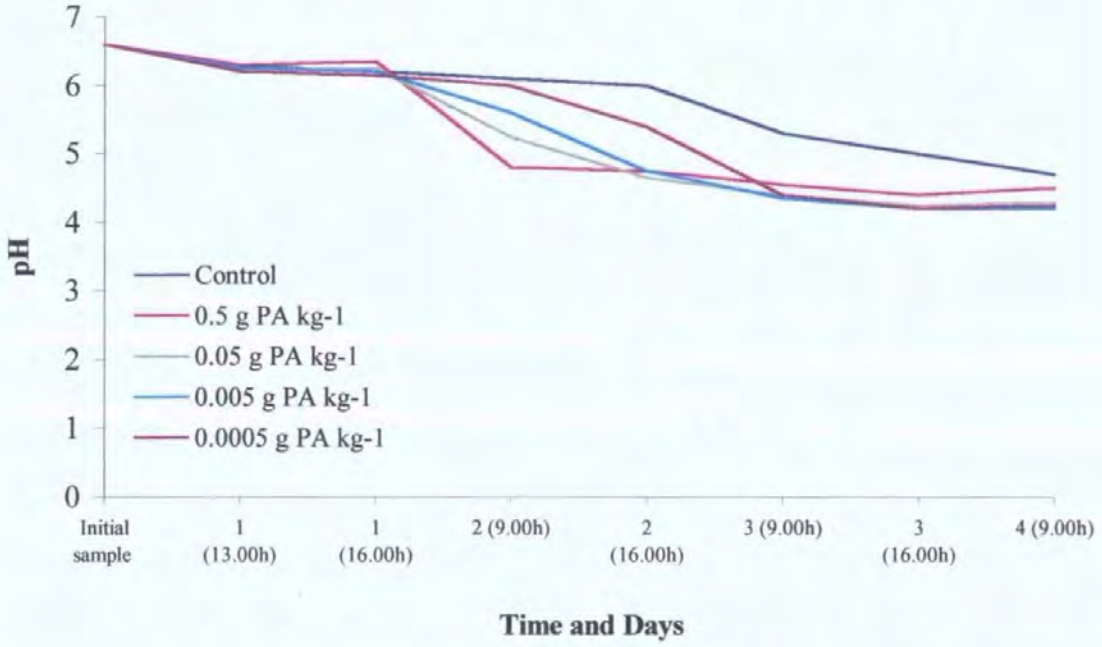
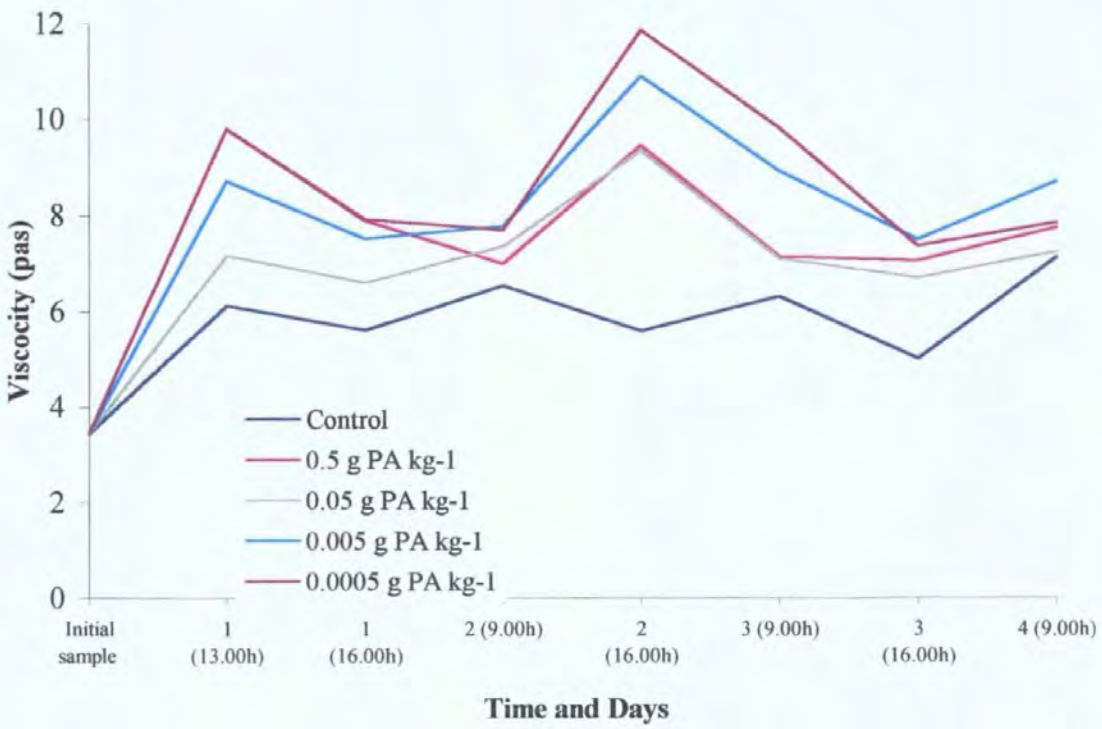


Figure 5.4 The effect of graded additions of *Pediococcus adidlactici* (PA) on the viscosity of potato steamed peelings.



5.7.2 Treatments Part 2

The development of the microbial populations was different between treatments (**PSPN** and **PSPPA**). In the control treatment (**PSPN**) there were no coliform bacteria although a natural population of lactic acid bacteria did develop within 48 hours (Figure 5.5 a,b). The high counts in the control treatment (**PSPN**) indicated that other species of microorganisms were present. The control treatment (**PSPN**) was also observed to be foul smelling compared to the inoculated treatment (**PSPPA**) which was not unpleasant. The inoculated treatment (**PSPPA**) was colonised by lactic acid bacteria within 48 hours and remained stable over time (Figure 5.5 a,b) and there were no coliform bacteria present. The increase in lactic acid bacteria resulted in a rapid reduction in the pH of PSP from 6.6 to 4.2 over time for both treatments **PSPN** and **PSPPA** (Figures 5.6 a,b).

Figure 5.5 Total microbiology of potato steamed peelings.

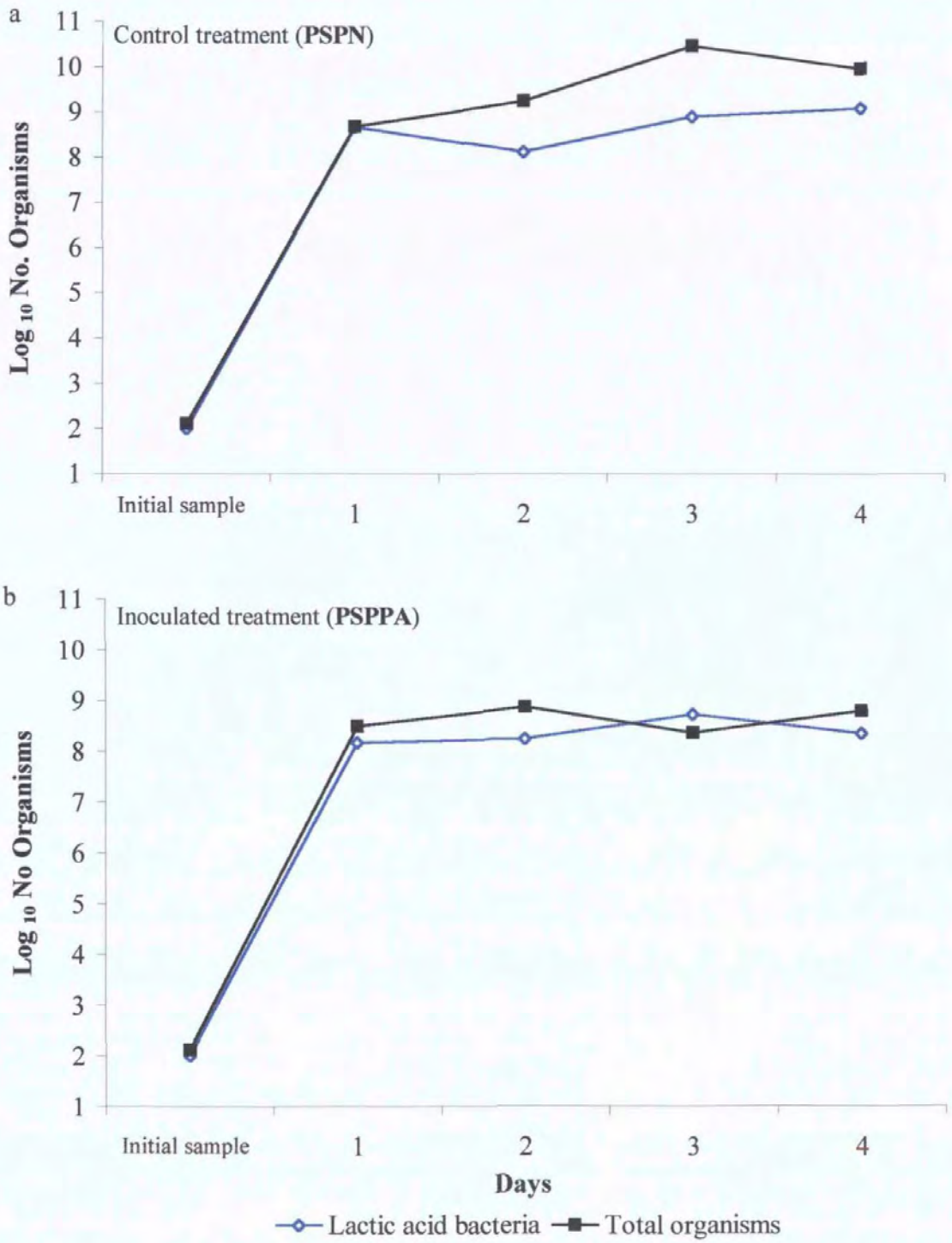
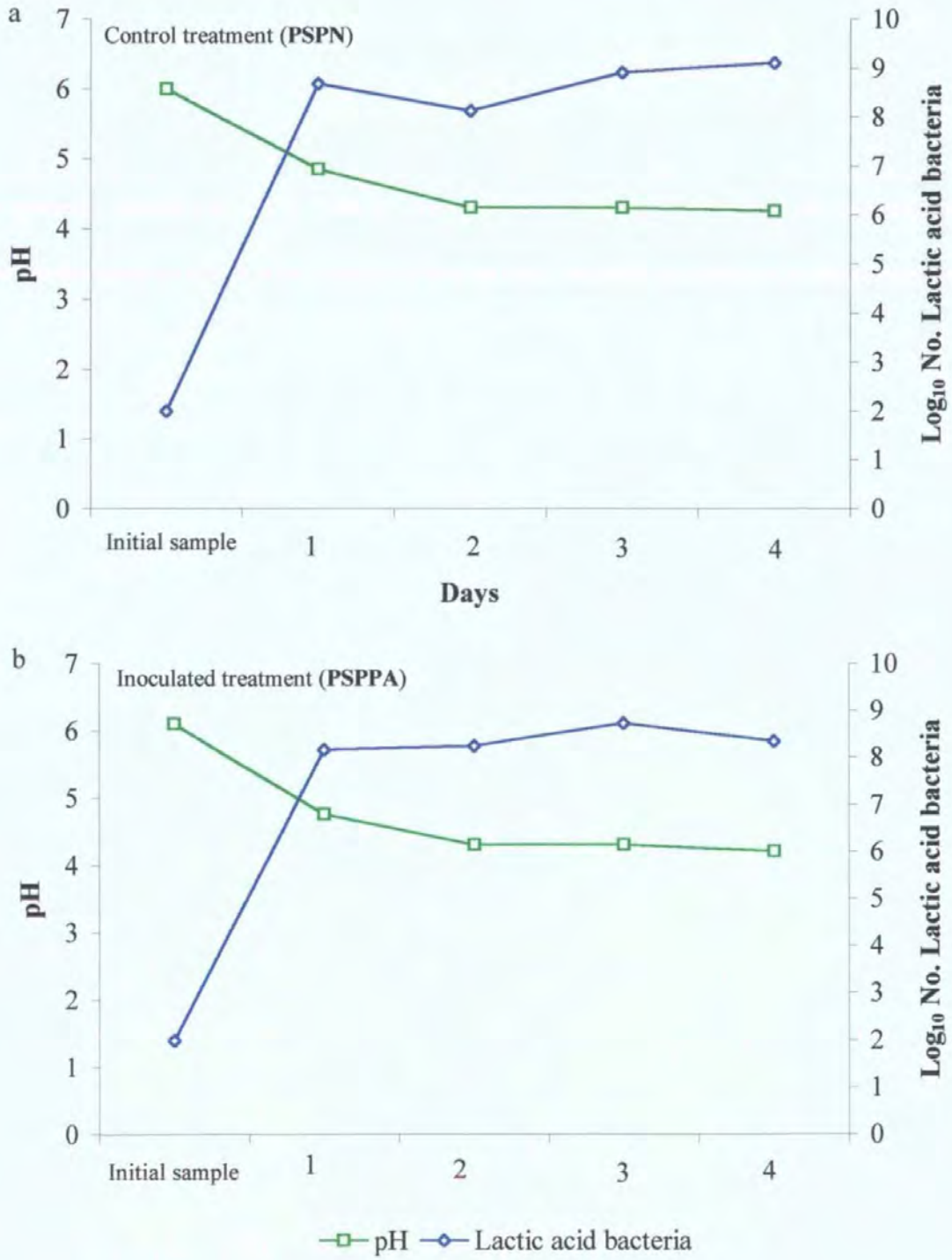


Figure 5.6 The relationship between lactic acid bacteria and pH in potato steamed peelings.



5.8 Discussion and Conclusions

In potatoes starch is present in the form of granules which are difficult to digest. Heating to 60°C gelatinises the starch, making it more digestible. Some of the gelatinised starch re-associates forming retrograded starch, which may then be less digestible, (Reid, Hillman, Henderson and Glass 1996). The process of gelatinisation increases the viscosity of the system. When potatoes are cooked in water above 60°C partial gelatinization of the starch occurs (Reid *et al.* 1996). The PSP used in this experiment were subjected to boiling and cooling in water which would have resulted in gelatinization and the production of some retrograded starch. Most of the starch which the pig consumes is hydrolysed and absorbed in the small intestine, however some starch will pass undigested into the colon of the pig where it is fermented by anaerobic bacteria to produce volatile fatty acids, hydrogen, carbon dioxide and sometimes methane (Reid *et al.* 1996).

Drochner, Mayer and Rensing (1988) examined the effects on digestibility of feeding cooked potatoes (not fermented) to 40 - 50 kg pigs and found that most of the digestion of starch occurred in the small intestine. In their experiment the apparent digestibility of crude protein and nitrogen free extractive in cooked potatoes were found to be 85.0 and 98.1 respectively compared to raw potatoes 42.0 and 94.6. In older pigs, which have better developed digestive systems it might be expected that digestibility of cooked potato would be better.

In older pigs the microflora of the gut has developed to an extent where it will be a benefit to the pig, and it is known that the adult pig contains a species of anaerobic bacteria (*Clostridium butyricum*) in its caecum which is capable of fermenting raw potato starch to produce butyrate (Baker, Nasr, Morrice and Bruce 1950). Butyrate is a valuable energy source to the pig (Reid *et al.* 1996). In the newly weaned pig the microflora system is still

developing and it may not possess the correct balance of organisms to bring about caecal fermentation of retrograded starch, thereby wasting energy.

The results obtained in this study demonstrated that PSP provides a readily available substrate, not only for beneficial lactic acid bacteria, but also for undesirable spoilage microorganisms, some of which were found in the control treatment (**PSPN**). These microorganisms may have been present in the form of heat resistant spores (Moreau 1979). The palatability of the product was not tested on piglets, however, it might be expected that the pigs would reject the foul smelling material products.

Fermenting PSP with *Pediococcus acidilactici* did not produce any benefits from reducing the viscosity as demonstrated in all of the inoculated treatments. As viscosity can be used as a measure of starch hydrolysis, it could be concluded that little or no starch hydrolysis occurred in the inoculated treatments and therefore there would not be an increase in digestibility.

In this study inoculation with *Pediococcus acidilactici* (**PSPPA**) produced an active fermentation which was a result of the inoculant and not yeasts since the material had been heated to greater than 52°C (the temperature needed to kill yeasts) (Bouix and Leveau 1995). The study demonstrated that inoculation of PSP with a lactic acid bacterium can provide a measure of control over the fermentation pattern achieved. pH was lowered, providing a degree of biosecurity although further studies are needed to assess the effect of fermentation treatments on palatability and digestibility.

EXPERIMENT 7. CONTROLLING THE FERMENTATION OF FOOD INDUSTRY LIQUID RESIDUE DIETS BY THE USE OF INOCULANTS

5.9 Introduction

Lactic acid fermentation can be used as an important tool in preserving foods. In the human food industry processing inoculum recycling or 'back slopping' is employed. During this process the microflora developing in a fermentation is inoculated into the next batch of food (Dillon and Cook 1994). This system could be equally well employed in a liquid feed system for pigs and could provide greater biosecurity in the form of inhibition of undesirable pathogenic and spoilage microorganisms. There are very few published experiments on the use of lactic acid bacterial inoculants for use as preservatives in food industry liquid residue (FILR) diets for pigs. Those few papers which have been published are concerned mainly with the preservation of either poultry offal (Tibbetts *et al.* 1987; Urlings, Bijker and Van Logtestijn 1993) or fish viscera (Ahmed, Ramesh and Mahendrakar 1996 ; Rose *et al.* 1994; Owens and Mendoza 1985). Urlings *et al.* (1993), preserved poultry offal by inoculating it with *Lactobacillus plantarum*. Their results showed that pigs fed on a diet, which had been fermented with the inoculant, had significantly better feed conversion rates (2.46 *vs* 2.57) and developed a significantly different gut microflora than pigs fed a compounded pig feed.

It has been reported that there are several beneficial effects of consuming lactic acid bacteria or fermented products. These include; stabilization of the gut flora; protection against pathogenic microorganisms; enhanced availability of protein and vitamins (Urlings *et al.* 1993). Lactic acid bacteria produce a wide range of antimicrobial metabolites, including organic acids, hydrogen peroxide, alcohols, diacetyl and bacteriocins. Many of these compounds are known to kill or suppress the growth of food-borne pathogens and

spoilage organisms (Dillon and Cook 1994).

In the previous experiments in this study inoculants were used in both compound liquid feeds and in potato co-product liquids in an attempt to control the pattern of fermentation and provide greater biosecurity. Producers feeding pigs liquid diets are currently using a variety of novel combinations of (FILR) as well as compound diets mixed with water. Many of the producers feeding pigs on liquid diets are experiencing patterns of uncontrolled fermentation in their liquid diets, most of these fermentations are beneficial but some are undesirable, because the pigs find the food unpalatable (J. Bell personal communication 1996). It is essential for producers to have control over the pattern of fermentation in order to preserve the diets and promote good health and growth performance from their pigs.

The objectives of this experiments were:

- to compare the fermentation patterns in three liquid diets containing FILR with or without the addition of two different inoculants
- to examine changes in the microflora of these diets over time
- to examine changes in the composition and nutritional value of the diets as a result of fermentation

5.10 Materials and Methods

5.10.1 Experimental design and treatments

The experiment was conducted using four diets. These comprised a basal diet (**B**) formulated from conventional dry feed components and three diets formulated to the same nutrient specification but each substituting a liquid food industry residue. The FILR used were Greenwich Gold (**G**), 'C'-Starch (**S**) and Whey (**W**). Each diet was either allowed to

develop a natural microflora (**N**) or was inoculated with *Pediococcus acidilactici* (**PA**) or *Enterococcus faecium* (**EF**). The twelve codes are summarised in table 5.4.

Table 5.4 Identification codes for treatments used in Experiment 7.

Diet	no inoculant (N)	*Pediococcus acidilactici* (PA)	*Enterococcus faecium* (EF)
Basal (B)	**BN**	**BPA**	**BEF**
Greenwich Gold (G)	**GN**	**GPA**	**GEF**
C-Starch (S)	**SN**	**SPA**	**SEF**
Whey (W)	**WN**	**WPA**	**WEF**

5.10.2 Diet formulations

The diets were formulated to provide 16 MJ DE kg^{-1} and 1.5% lysine at 87% DM equivalent. The composition of the diets used is given in table 5.5.

Milk whey was obtained from Curworthy Cheeses (Okehampton, Devon), collected (4 hours journey time) and stored at 4°C for not more than 24 hours before use. Greenwich Gold and C-Starch were supplied by Tunnel Refineries (Greenwich, London, UK), dispatched by Parcel Force (12 hour journey time), collected and stored at 4°C for not more than 24 hours before use.

Table 5.5 Declared nutrient composition of the diets used in Experiment 7.

Feedstuff	Basal	Whey	C-Starch	G-Gold
		Quantity		
Water	2332.1	919.90	1836.40	2029.60
Whey[a]		1500.00		
C-Starch[b]			600.00	
G-Gold[b]				400.00
Wheat[c]	219.71	172.22	146.50	151.16
Cooked wheat[c]	202.50	202.50	202.50	202.50
Cooked maize[c]	151.87	151.87	151.87	151.87
Skimmed milk powder[c]	151.87	151.87	151.87	151.87
Low temperature fish[c]	101.25	101.25	101.25	101.25
High protein soya[c]	60.75	42.82	25.51	32.70
Wheat feed[c]	50.62	35.43		50.62
Corn oil[d]	46.57	41.31	28.55	46.57
Di-calcium phosphate[c]	10.30	9.20	11.80	8.80
Lysine[c]	2.70	2.10	2.40	3.00
Threonine	1.50	1.20	1.60	1.50
Tryptophan	.18	.149	.248	.306
Methionine-DL	.08		.032	.159

[a] Obtained from Curworthy Cheeses (Okehampton, Devon)
[b] Tunnel Refineries (Greenwich, London, UK)
[c] I.S.C.A. Agriculture (Exeter, UK)
[d] Obtained from a local supermarket
[e] Sigma Aldrich Co. Ltd (Dorset, UK)

5.10.3 Preparation of food industry liquid residue diets

The dry feed components raw materials, wheat, cooked wheat, cooked maize were milled in a hammer mill with a 1.5 mm screen before mixing. The dry raw materials and oil, of the dietary treatments, were mixed together. They were then weighed into 600 ml beakers to which the appropriate amount of water and food industry liquid residues were added to form a substrate of 500 ml to provide the proportions described in table 5.5. Treatments were inoculated, and allowed to ferment at room temperature for a period of 5 days. The bacterial inoculants were added at a rate of 0.05 g to 1 kg of dry matter. A microbiological assessment was conducted together with a measurement of pH for each treatment.

The inoculant *Pediococcus acidilactici* (MA 18-5M) was supplied in freeze dried form, by Park Tonks Ltd (Cambridge, UK). The inoculant *Enterococcus faecium* (M74) (EF) was supplied in freeze dried form, by Medipharm (Kagerod, Sweden), both inoculants were stored at 4°C in a refrigerator before use. Both inoculants were tested to check viability and whether they would grow on the prepared agar under the conditions described below.

5.10.4 Microbiological assessment

A microbiological assessment was made of the liquid diets for each treatment, and the pH of each sample was recorded daily with a ph meter (Kent E1L 7015). In addition an initial microbiological assessment was made of the LIFR, Whey, C-starch and Greenwich Gold. The samples were plated within 2 hours of collection using the techniques described in Chapter 2, 2.2.1, with the following exceptions. Total Plate Count agar was replaced with Glucose Agar (Unipath Ltd). *Enterococcus faecium* was cultured on M-Enterococcus Agar (Difco Laboratories Ltd), using the spread plate method, incubated at 37°C anaerobically for 48 hours. *Pediococcus acidilactici* was cultured on S-L Acetate Agar (Difco Laboratories Ltd) using the spread plate method, plates were then incubated anaerobically at 44°C for 48 hours.

5.10.5 Proximate analysis of samples

Samples of 30 ml were withdrawn from each of the treatments initially and at the end of the experiment, and stored in a freezer in 100 ml plastic containers until analysis. The complete batch of samples were taken in their frozen state, in a cool box, and delivered to Frank Wrights Laboratory (Ashbourne, Derby) within 6 hours where a proximate analysis was conducted.

5.11 Results

5.11.1 Microbiology

The inoculants were found to be viable and did grow on their specifically designed agar substrate. Whey and C-Starch were found to contain organisms which grew on most of the treatment agar, however, Greenwich Gold did not contain organisms which would grow on the treatment agar (Table 5.6).

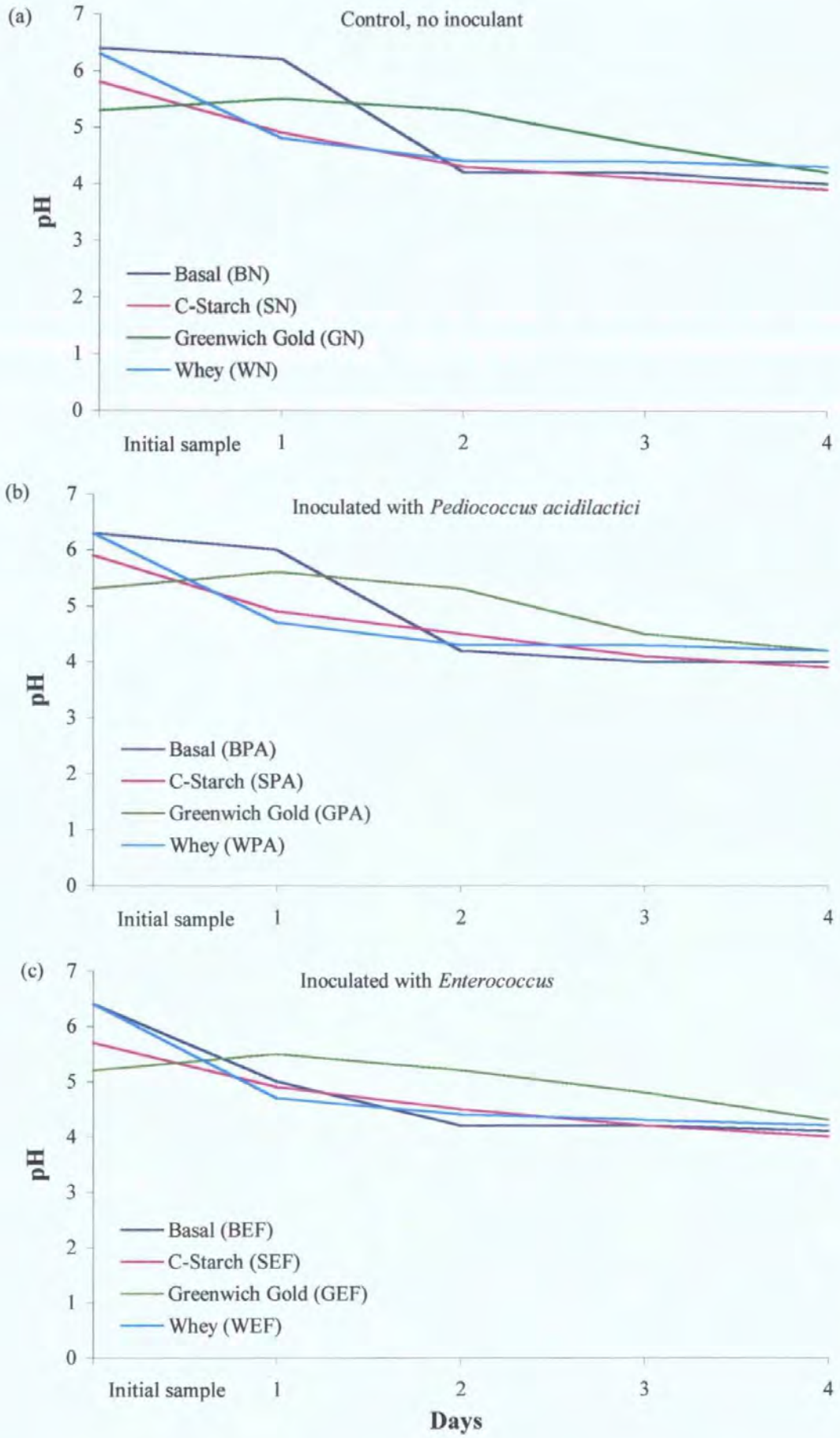
Table 5.6 Log $_{10}$ organisms present in whey, C-Starch and Greenwich Gold.

	Culture medium					
	MCC[a]	MRS[b]	M-E[c]	S-L[d]	RBCA[e]	GA[f]
Whey	0	6.49	0	2.47	2.69	6.16
C-Starch	0	8.57	6.17	7.95	7.01	6.92
Greenwich Gold	0	0	0	0	0	0

[a] MacConkey agar;
[b] de Mann, Rogasa and Sharpe, agar;
[c] M-Enterococcus agar;
[d] S-L Acetate agar;
[e] Rose Bengal Chloramphenical agar;
[f] Glucose agar.

The changes in pH of the treatments over time are presented in figures 5.7 a,b,c. There was a significant difference ($P<0.001$) in the initial pH between diets (Table 5.7). However, inoculation with either *Pediococcus acidilactici* or *Enterococcus faecium* had no significant effect on the final pH of any of the treatments (Table 5.7) which was never less that 3.9.

Figure 5.7 A comparison of the changes in pH over time in food industry liquid residue diets inoculated with either *Pediococcus acidilactici* or *Enterococcus faecium*.



The basal diet inoculated with *Enterococcus faecium* (**BEF**) produced a more rapid decrease in pH from 6.4 to 5.0 within 2 days than the basal diet without any inoculant (**BN**) (pH 6.4 to 6.2) or the diet inoculated with *Pediococcus acidilactici* (**BPA**), (pH 6.4 to 6.0). There was very little difference in the pH between any of the whey based diets (**WN, WPA** and **WEF**) which were reduced rapidly over time from *circa* pH 6.4 to 4.2. There was very little difference in the changes in pH between all the C-Starch based diets (**SN, SPA** and **SEF**) which were reduced rapidly over time from *circa* pH 5.9 to 4.0. The pH changes of the Greenwich Gold diets (**GN, GPA** and **GEF**) were different from all other treatments; pH increased after day 2 from *circa* pH 5.2 to 5.6 then decreased slowly to reach a final pH of *circa* 4.3 to 4.2.

All the treatments initially contained or developed a natural microbial population which contained lactic acid bacteria, and yeasts spp. There were no coliform bacteria in the initial samples taken from the basal, C-Starch and whey based diets. However, coliforms were present initially in low numbers in one of the Greenwich Gold treatments (**GPA**). After 24 hours there was a rapid increase in coliform numbers in all of the basal diets (**BN, BPA** and **BEF**). There was a significant ($P<0.01$) effect of diets on coliform numbers by day 5, which resulted in a complete elimination of coliform bacteria in all of the C-Starch and whey based diets. All the C-Starch based diets (**SN, SPA** and **SEF**) initially contained a high level of yeast spp. which were significantly different ($P<0.001$) from all other diets, and also a very high level of lactic acid bacteria which was significantly different ($P<0.05$) from the Greenwich Gold and basal diets. All the Greenwich gold and whey diets (**GN, GPA, GEF, WN, WPA** and **WEF**) developed populations of yeast spp. which were still continuing to grow over time. All the whey diets (**WN, WPA** and **WEF**) contained a high level of lactic acid bacteria which increased initially and then remained stable over time (Table 5.7)

Table 5.7 Comparison of dietary effects on the populations of microorganisms and pH as a result of fermentation.

Parameter	Diets (mean value) Basal	C-Starch	Greenwich gold	Whey	s.e.d.	
Day 1						
pH	6.37^{ac}	5.80^{cde}	5.27^{abd}	6.33^{be}	.06	***
Total organisms	4.49^{ac}	8.60^{ab}	5.20^{b}	7.01^{c}	.65	**
Lactic acid bacteria	4.15^{b}	8.93^{ab}	3.46^{a}	7.03	1.20	*
Coliform bacteria	0	0	.66	0	0.47	
Yeast spp.	2.88^{cd}	6.83^{abc}	1.43^{ad}	2.69^{b}	.36	***
S-L agar counts	2.74	8.89	3.42	4.60	2.53	
M-E agar counts	3.59	7.16	4.29	3.52	1.12	
Day 5						
pH	4.03^{cd}	3.93^{ab}	4.23^{ad}	4.23^{bc}	.04	***
Total organisms	8.57	8.60	8.83	8.35	.15	
Lactic acid bacteria	8.43	8.61^{a}	8.57^{b}	7.92^{ab}	.19	*
Coliform bacteria	4.85^{abc}	0^{b}	1.28^{c}	0^{a}	1.00	**
Yeast spp.	6.49	7.28	6.57	7.34	.49	
S-L agar counts	7.53	8.19	8.10	5.84	.71	
M-E agar counts	8.15	7.05	5.08	6.83	1.83	

Means with the same superscript in the same row differ significantly ($P<0.05$).
* $P<0.05$; ** $P<0.01$; ***$P<0.001$.

Inoculation with *Pediococcus acidilactici* did not reduce coliform bacteria populations compared with the control treatments (**BN, SN, GN** and **WN**) and had no significant effect on the final populations of lactic acid bacteria or yeasts spp. Inoculation with *Enterococcus faecium* did not significantly reduce coliform bacteria populations overall compared with the control treatments (**BN**, and **WN**). However, it was observed that inoculation with *Enterococcus faecium* did result in an absence of coliform bacteria in treatments (**SEF** and **WEF**) compared to the control treatments (**SN** and **GN**). Inoculation with *Enterococcus faecium* had no significant effect on the final populations of lactic acid bacteria or yeast spp. A comparison of the effects of inoculation on the populations of microorganisms and pH after 5 days of fermentation is given in table 5.8.

Table 5.8 Comparison of the effects of inoculation on the populations of microorganisms and pH after 5 days of fermentation.

Parameter	No inoculant	Inoculation with *Pediococcus acidilactici*	Inoculation with *Enterococcus faecium*	s.e.d[a]
pH	4.10	4.07	4.15	.35
Total organisms	8.50	8.52	8.73	.13
Lactic acid bacteria	8.35	8.46	8.33	.13
Coliform bacteria	1.46	2.30	.84	.86
Yeast spp.	6.87	6.74	7.14	.43
S-L agar counts	6.99	8.12	7.14	.61
M-E agar counts	5.30	7.47	7.57	1.58

[a] No significant effect for any treatments.

5.11.2 Proximate analysis

The expected and actual nutrient composition of the diets is given in table 5.9.

Table 5.9 Formulated and analyzed composition of diets expressed on 100% dry matter basis.

Component	Expected[a]	Basal Actual[a]	Whey Actual[a]	C-Starch Actual[a]	G-Gold Actual[a]
Energy (MJ DE kg^{-1})	17.77	15.78	16.02	15.58	15.90
Crude protein %	24.45	24.27	23.68	25.40	24.86
Oil %	7.54	5.26	5.85	4.13	6.14
Ash %	6.75	5.25	5.48	5.45	5.85
Crude fibre %	2.03	2.24	1.64	1.99	2.06
Nitrogen free extractive %		62.96	63.34	63.01	61.08

[a] calculated on mean values;

In all diets the energy, oil, ash and crude fibre components were very similar to the expected formulated diet. The crude protein level in all diets was slightly higher than had been predicted. In order that the differences in nutrient composition could be compared before and after treatment and to account for the moisture loss due to evaporation, the

values were corrected to a constant ash content on the basis that the ash content would not change over time. The differences in nutrient composition due to treatment are given in table 5.10.

A difficulty encountered in interpreting proximate analysis data from liquid diets is that there is some loss of moisture by evaporation over time. Consequently, nutrient values expressed as a proportion of the total may not reflect the true change in nutrient content. As the mineral content is unaffected either by evaporation or microbial action the diet analyses were recalculated and expressed on the basis of constant ash content. This corrected data (Table 5.10) shows that considerable moisture was lost from the system (range 8.24 - 18.14%). Crude protein was little changed. However, this does not provide a complete picture as there may have been changes in amino acid content as a result of incorporation of feed protein into microbial protein. The changes in dry matter are largely reflected in the changes in nitrogen free extractive. This is as expected as the nitrogen free extractive faction would include the sugar which will have been used as a substrate by the developing microflora.

In treatments **WN**, **WPA** and **WEF** there was a larger loss of dry matter compared with all other treatments. Treatment had little effect on crude protein, crude fibre and oil. However, there was a decrease in the nitrogen free extractive in treatments **WNC**, **WPA** and **WEF** compared to the all other treatments. There were changes within diets as a result of adding inoculants for example; treatment **BN** had a higher loss of oil and nitrogen free extractive than treatments **BPA** and **BEF**; treatment **SN** had a greater loss of crude protein compared to treatments **SPA** and **SEF**; treatment **WN** had a higher loss of dry matter and nitrogen free extractive compared to **WPA** and **WEF**.

Table 5.10 Changes in composition of diets following 5 days fermentation g kg^{-1} (corrected to constant ash content)

Diet	Basal			Greenwich Gold			C- Starch			Whey		
Inoculant	N	PA	EF	N	PA	EF	N	PA	EF	N	PA	EF
Dry matter	-3.4	+10.3	+11.7	+30.5	+16.9	-13.2	+7.9	+13.4	+9.1	-43.1	-29.2	-31.9
Moisture	-130.9	-134.3	-136.8	-82.4	-94.1	-98.4	-119.5	-181.9	-113.5	-123.5	-143.8	-160.5
Crude protein	+2.3	+1.0	+3.0	+2.1	+4.8	-4.1	-5.8	+2.6	+2.0	+0.7	0	-0.6
Oil	-1.4	+0.5	0	+0.1	+0.7	+0.8	+1.2	0	+0.3	+1.0	+1.6	0
Crude fibre	+0.6	0	+0.5	+0.6	-0.5	-0.1	-0.3	-0.7	+0.3	0	0	0
Nitrogen free extractives	-5.0	+8.8	+8.2	+27.6	+11.8	-9.9	+12.8	+11.5	+6.5	-44.8	-30.8	-31.2

N = No inoculant;
PA = Inoculated with *Pediococcus acidilactici*;
EF = Inoculated with *Enterococcus faecium*;
\+ indicates an increase in component;
\- indicates a decrease in component

5.12 Discussion and Conclusions

All the LFIR diets were microbiologically active from the outset of the study and the populations of microorganisms, particularly lactic acid bacteria and yeast spp. increased over time (Figures 5.8 a,b,c,d, 5.9 a,b,c,d and 5.10 a,b,c,d). The final pH of all diets was between 3.9 and 4.3 (Figure 5.7 a,b,c). This is below the optimum pH range for the growth of food borne pathogenic organisms (*Escherichia coli*, *Salmonella* (most), *Clostridium perfringens* and *Staphylococcus aureus*) (Banwart 1989). This fact alone demonstrates that the controlled use of inoculants can provide a greater degree of biosecurity in both basal diets mixed with water and liquid food industry residue diets. Dierick (1989) suggested that lactic acid bacteria can be used to convert food industry waste products into safer sources for livestock feeds whilst at the same time conserving feed energy. It was demonstrated in this experiment that in general reducing the pH to below 4.3 resulted in a reduction in coliform numbers in most of the treatments. However, inoculation with *Enterococcus faecium* was found to be more efficient than *Pediococcus acidilactici* in reducing coliform numbers in Greenwich Gold based diets (Figure 5.10 c) and was more efficient at reducing the pH of the basal diet (Figure 5.7 c). Both whey and C-Starch based diets were already very biologically active at the start of the experiment with large populations of lactic acid bacteria and yeast spp. Therefore, inoculation with either *Pediococcus acidlactici* or *Enterococcus faecium* made little difference to pH or coliform numbers in these products (Figures 5.9 a,b,c,d and 5.10 a,b,c,d).

Figure 5.8 Changes in the microbiology of non-inoculated liquid diets.

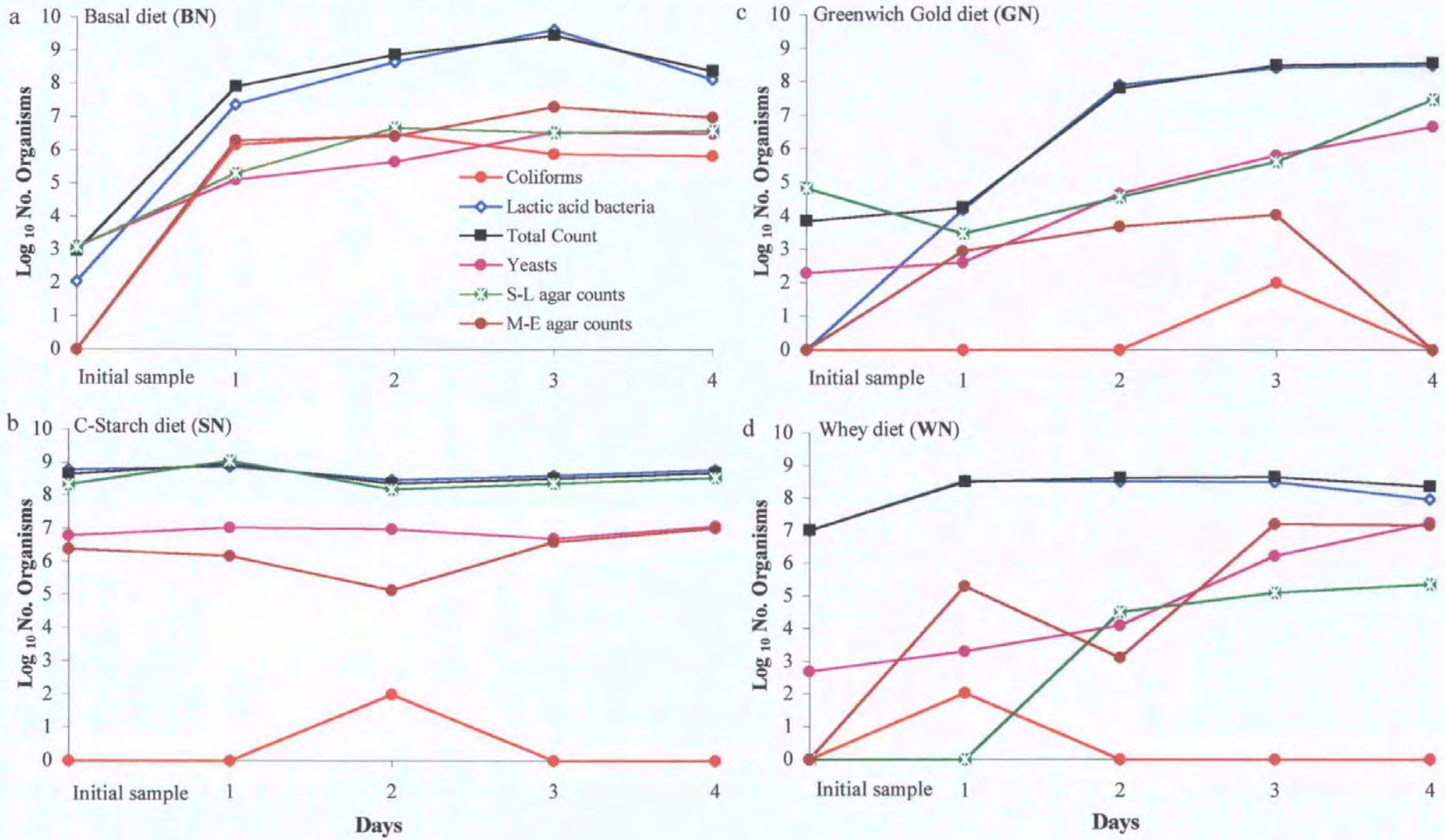


Figure 5.9 Changes in the microbiology of liquid diets inoculated with *Pediococcus acidilactici*.

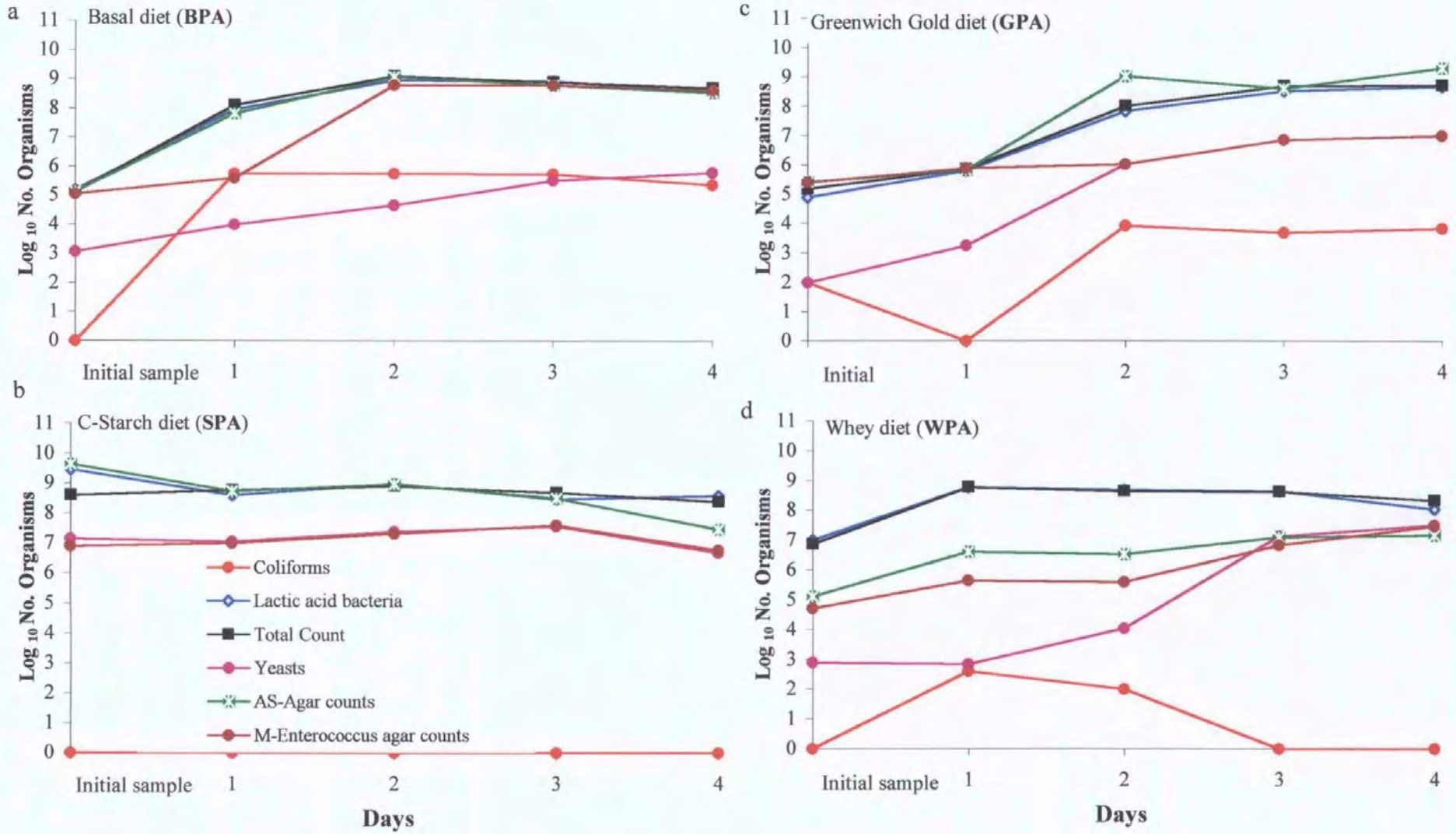
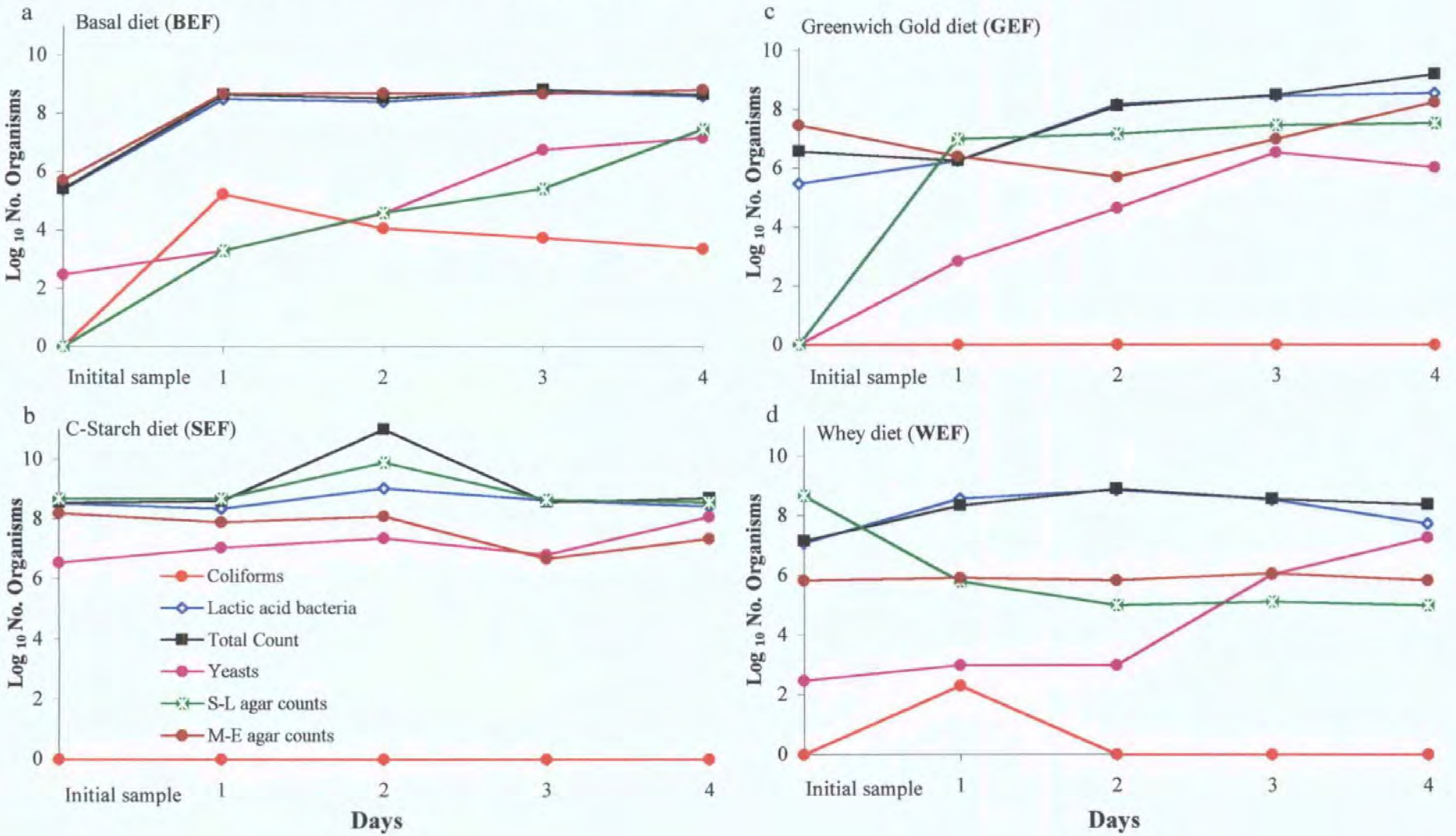


Figure 5.10 Changes in the microbiology of liquid diets inoculated with *Enterococcus faecium*.



The organisms which initiated the fermentation, whether they were naturally occurring or inoculated, would have developed until the by-products of their growth inhibited further growth and fermentation (Pederson 1979). In a closed system, such as in this experiment, it would only be a matter of days before this happened; however in a system such as a liquid feeding system, new material is constantly being added and this would continue to support the growth of microorganisms. There will always be a succession of microorganisms in a system allowed to ferment naturally. In general the growth of microorganisms will be initiated by bacteria, followed by yeasts (Inge Dorthe personal communication 1996) and finally by mould. One of the reasons for this is that the smaller the surface area of a microorganism the faster its rate of growth (Pederson 1979). In this experiment it was found that this happened to the extent that naturally occurring lactic acid bacteria increased rapidly followed by yeasts. In previous experiments in this study (Experiments 1, 2 and 4) a similar pattern of events occurred. Although a lactic acid fermentation is desirable it has to be one which is managed because natural fermentation in liquid feed systems designed for pigs can be detrimental as on farm experiences have shown (J. Bell personal communication 1996).

In a properly managed system, microbial metabolism is regulated primarily by lactic acid bacteria which not only produce preservative compounds (Dillon and Cook 1994) but lack the ability to degrade protein and oil (Inge Dorthe personal communication 1996). Controlled fermentation is achieved by adding very high numbers (10^6 cells per ml of viable and active cells) of desirable inoculant to the raw material and incubating it under optimum conditions (Ray and Daeschel 1992). It must be recognised that when bacterial inoculants are used in liquid feed systems for pigs the incubation conditions may not be optimal. The efficacy of a bio-preservative system will depend on; the bacterial inoculant; the type and concentration of fermentable carbohydrate; the initial pH and buffering

capacity of the diet; the nutritional profile of the diet; factors inhibiting the bacterial inoculant; growth characteristics of the bacterial inoculant; (Dillon and Cook 1994).

There have been a large number of experiments which have examined the use of lactic acid bacteria to preserve or inhibit pathogens in foods destined for human use (Jay 1982; El-Gendy, Abdel-Galil, Shahin and Hegazi 1983a; Adams and Hall 1988; Daeschel 1989; Spelhaug and Harlander 1989; Berry, Hutkins and Mandingo 1991; Juven *et al.* 1991; Tharrington and Sorrells 1992; Stiles 1994; Tarelli, Carminati and Giraffa 1994; Cintas, Rodriguez, Fernandez, Sletten, Nes, Hernandez and Holo 1995; Einarsson and Lauzon 1995; Itoh, Fujimoto, Kawai, Toba and Saiton 1995; Olsen, Halm and Jakobson 1995). However, only a few experiments have examined their use for preserving or inhibiting pathogens in liquid feed diets for pigs (Owens and Mendoza 1985; Tibbetts *et al.* 1987; Urlings *et al.* 1993; Rose *et al.* 1994; Ahmed *et al.* 1996). Daeschel (1989) suggested that the intentional use of lactic acid bacteria in the production of fermented foods for human use has long been considered a safe process because of the lack of any evidence to the contrary. This has been tested on pigs to a certain extent where lactic acid bacteria have been given as direct oral doses with the intention of improving their health (Muralidhara *et al.* 1977; Pollman *et al.* 1980; Ozawa, Yokota, Kimura and Mitsouka 1981; Underdahl, Torres-Medina and Doster 1982; Ozawa, Yabu-uchi, Yamanaka, Yamashita, Nomura and Oku 1983; Lessard and Brisson 1987; Apgar, Kornegay, Lindemann and Wood 1993; Havenaar and Huis in't Veld 1993; Scheuermann 1993).

Lactic acid bacteria can convert carbohydrates to lactic acid with only minor changes in nutrient composition. There is little caloric change in the conversion of carbohydrates to lactic acid and very little loss of nutritive value (Pederson 1979). In this experiment the changes in nutrient composition as a result of fermentation with lactic acid bacteria were

examined (Table 5.10). To the experimenters knowledge there are no published papers which have examined the changes in nutrient composition of FILR diets as a result of fermentation with or without lactic acid bacterial inoculants. The main observation concerning changes in nutrient composition was that in all treatments there was a considerable loss of moisture as a result of fermentation and that there was a higher loss of dry matter in all of the whey based diets compared to all other treatments. As might be expected there was very little change in the levels of crude protein and oil as a result of fermentation. In diets where microbial activity of naturally occurring lactic acid bacteria and yeast spp. was initially high (**WN**, **WPA** and **WEF**) there was a loss in nitrogen free extractive which would have been used as the energy source for these organisms. Although lactic acid bacteria would appear to be able to provide many benefits to a liquid feed system, there can be some disadvantages if the wrong inoculants are selected. For example, some lactic acid bacteria have undesirable properties and can act as spoilage organisms. A particular undesirable metabolic property is the production of biogenic amines (histamine, tyramin, putrescine and cadaverine) which can result in a lower feed conversion ratio in farm animals (Brink and Huis in't Veld 1991). For this reason it would be better to select inoculants which are amino acid decarboxylase negative.

Many FILR lend themselves to upgrading and if it were not for the use the animal feed industry makes of these products they would create a major pollution problem (Perry 1995). The antimicrobial systems produced by lactic acid bacteria offer great potential for the development of effective natural preservation methods for use in foods (Earnshaw 1992) and can be used effectively in FILR diets for pigs. However, a great deal more research is needed to ensure that the correct lactic acid bacterial inoculants are selected for use on an increasing array of FILR so that biosecurity can be built into what is a potentially a very useful approach to the feeding of pigs.

CHAPTER 6

CONCLUDING DISCUSSION

In the past liquid feeding of weaner pigs has not been very successful. This was mainly because liquid feed systems had not been designed in a way which could deliver the food in a hygienic and palatable state (English *et al.* 1988). Where liquid feeding has been tried it was necessary to use elaborate cleaning techniques in order to sterilise the equipment between feeds to prevent microbial contamination: a process which was very expensive and labour intensive. Even if this was done there would still have been a risk to the piglet because any highly nutritious liquid substrate will become contaminated with spoilage microorganisms within minutes of mixing. These spoilage microorganisms will multiply rapidly in the warm environment of the weaner house and contaminate the feed which then sours and becomes unpalatable and microbiologically unsafe for newly weaned piglets. This is why liquid feeding systems have failed to gain acceptance as a system for rearing newly weaned piglets in the past.

The success of the liquid feeding system used in this study has been achieved because the liquid diets were allowed to ferment naturally or through inoculation with lactic acid bacteria before being fed to the weaner pigs. The technique of using lactic acid fermentation was a vitally important factor in the success of rearing newly weaned piglets in this study. The most important benefit of fermenting liquid diets is that this technique maintains the food in a hygienic and palatable state which provides a greater degree of biosecurity for the piglet. This is because the acidity of the fermented diets, (when they are below pH 4.2) and the anti microbial compounds which are produced, reduce the activity of food borne pathogenic organisms. In particular *Escherichia coli*, *Salmonella* (most), *Clostridium perfringens*, *Staphlococcus aureus* will be reduced (Banwart 1989) which means that the food can then be fed continuously to the piglet.

It was reported earlier in this study that the piglet encounters a microbial challenge at weaning which often leads to coliform scours (McAllister *et al.* 1979; Nabuurs 1995). The pre-weaned piglet possesses an intrinsic mechanism whereby lactose in sows's milk is converted to lactic acid by the microflora in its stomach. Weaning to dry feed disrupts this process. However, the lactic acid fermented diet supplements the intrinsic mechanism which the suckling piglet possesses; therefore in theory, the indigenous beneficial microflora of the piglet should be less disrupted by this technique. It is known that compound dry diets contain an indigenous coliform bacteria (Chapter 4, Table 4.5) and that the form of the diet can influence the indigenous microflora of the pig (Krause *et al.* 1995). These microorganisms can thrive on any undigested substrate which may pass into the digestive system of the piglet (especially when they are fed on dry diets which have a high buffering capacity) and this proliferation of coliforms may result in scours (Bolduan *et al.* 1988). Feeding fermented liquid diets can overcome this problem and reduce the possibility of coliform scours. Mounting an immune response has been shown to be costly in terms of energy to the animal (Stahly 1996). This being the case it follows that the less energy the piglet has to expend fighting off disease challenge, the more energy it will have to convert to lean tissue growth. It must be recognised that this technique will only work if the liquid diet is allowed to ferment long enough to acidify the diet to a pH of approximately 4.0. In this study the time taken to achieve a pH of approximately 4.0 (using compound weaner diets mixed with water) varied from 4 to 7 days (Experiments 1, 2, 3 and 4). When liquid food industry residues are substituted in the diets the outcome will be different (better or worse) due to the nature of their microbial populations (Chapter 5, Table 5.6).

The results of these studies demonstrated that when food was being continuously presented to the piglets, in a form which was hygienic, highly palatable, and resembled sow's milk

far more closely than dry feed, piglets were encouraged to start eating sooner and maintain their voluntary food intake. By avoiding the initial critical period of starvation (which newly weaned piglets on dry diets often encounter) the integrity of the gut can be preserved. Work with rats has shown that villus architecture is adversely affected by starvation (Steiner *et al.* 1968; Rudo *et al.* 1976). When the gut integrity of the piglet is preserved then the absorption and digestion of nutrients will be maintained, and the piglet will achieve a growth rate which approaches its genetic potential.

In the past researchers have attributed the problem of post weaning growth check to antigenic responses in piglet diets (Hall and Byrne 1980; Miller *et al.* 1984a; Miller *et al.* 1984b; Newby *et al.* 1985; Li *et al.* 1991) which they suggest contributes to the detrimental affects seen in the gut architecture of young pigs. However, when the data from these studies is re-examined it can be seen that this response may be attributed to nutrient deprivation, rather than to an antigenic response. This could explain why Kelly *et al.* (1990) and Hampson *et al.* (1988) failed to produce a hypersensitivity response to dietary antigens. In their studies piglets were force fed and as a result were maintaining an adequate nutrient intake which preserved their gut architecture. Re-examination of the data of Li *et al.* (1991) (presented in Chapter 1, Table 1.5) also clearly demonstrated that maintenance of gut architecture was a feature of feed intake and was not entirely due to an antigenic response. In their study piglets fed on milk protein had higher feed intakes 0 - 14 days post weaning (301 g d^{-1}) than those on soya protein concentrate (which contain antigenic compounds) which had feed intakes of only 251 g d^{-1}. As a result the piglets on the higher feed intakes (milk protein diets) had superior villus heights (364 µm) than the piglets on lower feed intakes consuming soya protein diets (234 µm).

During the course of this study research has been published by Pluske *et al.* (1996a) and

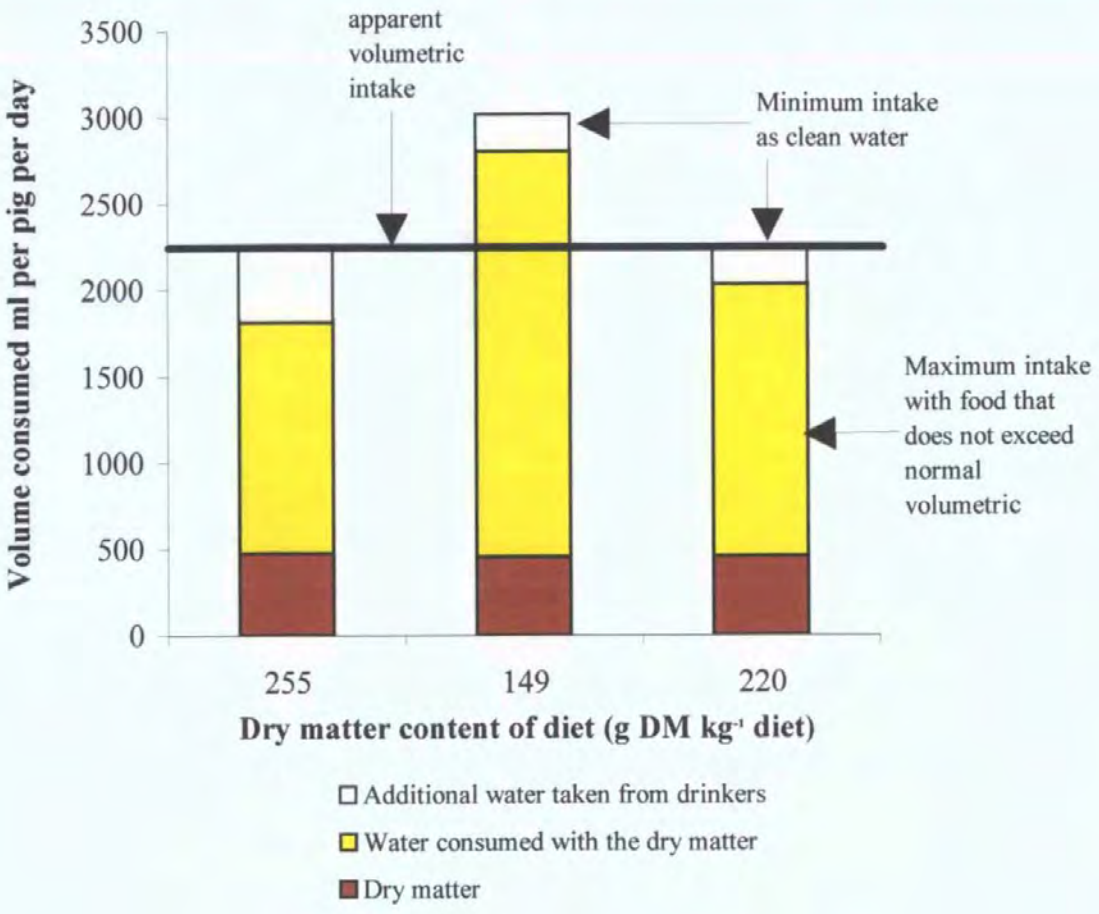
Pluske *et al.* (1996b) which supports the view that nutrient intake is a key factor in the success of rearing newly weaned piglets as found in the current study. Pluske *et al.* (1996b) demonstrated that maintaining voluntary food intake in the period 0 - 7 days post weaning is a critical factor in the preservation of the gut architecture (Chapter 1, Figure 1.5). The importance of maintaining voluntary food intake in newly weaned piglets cannot be emphasised strongly enough, for it is the key to success. This has very significant consequences, because nutrient intake in the first seven days post weaning, is the single most significant factor which affects growth performance 28 days later. In this study, by pooling the data for all liquid fed pigs, it was clearly demonstrated that feed intake 0 - 7 days post weaning had a highly significant effect ($P<0.001$) on 28 days post weaning weights. The consequences for the pig producer are that for every 10 grams of extra feed the young piglet can be encouraged to eat in the critical period seven days post weaning, 174 g of extra body weight will be accumulated by 28 days post weaning.

In a liquid feed system the nutrients are suspended in water and the pig has to be able to consume enough of this liquid to satisfy its nutrient requirements. However, voluntary food intake can be affected by a multitude of interacting factors (Chapter 1, Figure 1.10). When considering the factors which affect the voluntary food intake of newly weaned piglets on liquid diets, the most important factor is the extent to which volumetric fill limits nutrient intake. The concept of total volumetric fill was developed by Yang *et al.* (1981) and subsequently developed by Barber (1992). Yang *et al.* (1981) hypothesised that the regulation of voluntary food intake rises from abdominal fill. They demonstrated that water intake of growing pigs (about 30 kg liveweight) significantly increased when feed supply was abruptly reduced. Conversely when feed intake was suddenly increased water intake declined slightly or remained unchanged (Yang *et al.* 1981). Yang *et al.* (1981) proposed that growing pigs (30 kg liveweight) had a total volumetric intake which represented 19%

of their bodyweight. In subsequent studies Barber (1992) established that the total volumetric intake of older pigs (30 - 60 kg liveweight) was only 12% of their bodyweight. Barber (1992) suggested that newly weaned piglets do have a limited volumetric intake although this was not established in his study.

The total volumetric intake of newly weaned piglets has been established in this study. In experiment 2 it was established that the total volumetric fill of newly weaned pigs fed a liquid diet of 149 g DM kg^{-1} was 30% of their liveweight. This supports the study of Kvasnitski 1951 cited by Kidder and Manners (1978) who demonstrated that the gut capacity of the developing pig is increasing rapidly. In the past it may have been considered that newly weaned piglets lacked the gut capacity to take large volumes of liquid diets and this gave rise to the practice of feeding highly concentrated dry diets. However, this is not the case, and in the light of this study it can be demonstrated that weaner pigs can tolerate a wide range of dry matter concentrations in liquid diets and still maintain nutrient intake. However, there is an optimum dry matter concentration of liquid diets for weaner pigs which maximises nutrient intake without overriding the piglets mechanism for total volumetric fill. It was calculated from the data in this study that, in order to maximise nutrient intake without overriding the piglets mechanisms for total volumetric intake, pigs should be fed a liquid diets containing not less than 220 g DM kg^{-1} diet (Figure 6.1). Where piglets were fed on liquid diets containing 255 g DM kg^{-1}, the total volumetric fill was calculated as being 19% of body weight (Experiment 2) and 17% (average for all pigs in Experiment 4). From the data it can be shown that total volumetric fill is remarkably constant when weaner pigs are fed a fermented liquid diet containing 255 g DM kg^{-1}.

Figure 6.1 A model of the total volumetric intake of newly weaned pigs which are fed on liquid diets.



It was also reported in the literature review, that feed manufacturers acidify the diets of young pigs in order to overcome their lack of gastric acidity and hence reduced digestive capacity (Chapter 1, Table 1.13). When diets are fed fermented and in liquid form the pH is approximately 4.0 which is very similar to the gastric pH of a piglet aged between 20 and 30 days of age (Kershaw *et al.* 1966). Providing a diet with a low pH will supplement the piglet's lack of gastric secretion and enhance proteolytic digestion. It is also known that the presence of acid and food in the duodenum of the pig will stimulate the hormones secretin and cholecystokinin which in turn stimulate the pancreas to release pancreatic enzymes for the digestion of nutrients (McDonald *et al.* 1988). Acidified liquid diets will have distinct advantages over a dry diets (which have high buffering capacities), in activating this natural process. The process of fermenting the liquid diets will have resulted in diets which were in a more digestible form. This is because the action of soaking converts the starches in the cereal fractions to simpler sugars under the action of natural amylases (Lawrence 1982).

By making the transition from sow's milk as smooth as possible *(i.e.* feeding warm, liquid, easily digestible, acidified, frequent, well balanced diets) the process of weaning young piglets can be a highly successful practice.

Piglets clearly have the genetic potential to grow at an astonishing rate provided they are given the correct diet and conditions. It can be shown that a correctly managed liquid feed system results in piglets who are approaching their full genetic potential for growth. From the data presented in Experiment 4 it can be seen that piglets with the highest growth rates were capable of gaining 21.2 kg of weight in 28 days.

The data presented earlier (Chapter 4, Table 4.7) demonstrated that with each successive

Experiment the feed conversion efficiency of the piglets on liquid diets improved from 1.89 to 1.11. The Meat and Livestock Commission (1996), reported that their top third rearing herd were achieving a feed conversion efficiency of only 1.73 for dry fed pigs (MLC 1996) (Table 6.1). Therefore, it can be concluded that liquid feeding fermented diets can be superior to dry fed pigs provided they are managed correctly.

Table 6.1 Comparison of the performance of weaner pigs in this study with MLC top third rearing herd for 1996

Parameter	Exp 1[a] **DF**	Exp 1[b] **LF**	Exp 2[c] **(DM255)**	Exp 3[d] **(PA)**	Exp 3[e] **(LA)**	MLC
Start weight	6.8	6.7	7.0	8.0	7.7	6.4
Finish weight	18.0	19.4	18.3	21.3	21.6	36.7
Total gain	12.7	11.1	11.3	13.3	14.0	30.3
Feed conversion ratio	1.37	1.44	1.20	1.15	1.11	1.73

[a] Dry diet, Experiment 1, Trial 2;
[b] Liquid diet, Experiment 1, Trial 2;
[c] Liquid diet, dry matter concentration of 255g kg^{-1};
[d] Liquid diet, inoculated with *Pediococcus acidilactici*;
[e] Liquid diet, acidified with lactic acid.

There are several reasons why the feed conversion efficiency and consequently the growth of the piglets was superior in Experiment 4 of this study. It was calculated that the fermented liquid diets used in Experiment 4 contained a level of alcohol approximating 2 or 3% w/v (Chapter 4, Figures 4.5 a,b). It was theorised that this level of alcohol may have affected the behaviour of the piglets because they were observed to be less active and spent more time resting huddled close together, compared to piglets on dry diets in Experiment 1. A study was conducted using the same facilities as this study to examine the effects of feeding fermented liquid diets, dry diets and a choice of dry or liquid diets

on the behaviour of weaner pigs (Murray 1995). From her study Murray established that belly nosing (which may be considered an unfavourable behaviour) was significantly ($P<0.001$) reduced in liquid fed pigs compared with dry fed pigs. It was also established that liquid fed pigs spent a greater proportion of their time resting compared with pigs offered a choice of dry and liquid (Table 6.2). The data presented in Murray's study suggests that the behaviour of weaner pigs is modified by the diet, and that the fermented liquid diet improves their welfare.

Table 6.2 Effect of belly nosing for more or less than 1.5% of time on growth rate of weaners

	Belly nosing < 1.5% of time	Belly nosing > 1.5% of time
Proportion of time belly nosing	0.002 *	0.035
Proportion of time sleeping/resting	0.87 ***	0.82
Daily gain (g)	309 *	265
No. liquid fed pigs	14	4
No. dry fed pigs	9	9
No. preference fed pigs	7	11
Totals	30	24

(Brooks, Geary, Morgan and Campbell 1996)

As a result of the calming influence of the fermented liquid diet the weaner pigs wasted less energy in movement. Furthermore postural changes may have resulted in less surface heat loss (Plate 6.1).

It was demonstrated in the first Experiment (Trials 1 and 2) that the liquid feed system could be successfully used to rear newly weaned pigs, and that the pigs which were fed on a liquid diet had significantly better ($P<0.001$) growth rates and feed intakes ($P<0.001$) than dry fed pigs.

It was reported in the literature review that weaning age and weaning weight had very significant effects on the physiological development of the newly weaned pig and subsequent growth performance, and that the abrupt process of weaning to dry diets was detrimental to the piglet. It was also demonstrated in this study that weaning age and weaning weight are not totally independent of each other. Weaning pigs at 24 ± 2 days when their active immunity is developing rapidly, will help them to overcome some of the disease challenges which they are exposed to. It was also demonstrated that weaning weight was an extremely important factor which influenced post weaning growth performance, and that this factor was even more important than weaning age. The heavier the piglet at weaning the more physiologically capable it is to adapt to the changes expected of it. This explains partly why the piglets in Experiment 4 had better post-weaning growth rates than pigs on liquid diets in Experiment 1: they had heavier average weaning weights of 8.0 kg for **PA** and 7.7 kg for **LA** compared with 6.7 kg in Experiment 1. As a result of the heavier weaning weights, piglets in Experiment 4 were physically capable of consuming larger quantities of feed in the first 0 - 7 days post- weaning than their lighter counterparts in Experiment 1.

In addition to modifying dry diets the technique of lactic acid fermentation can be used to upgrade liquid food industry residues. In Chapter 5, of this study, it was demonstrated that potato wastes could be fermented to provide a greater degree of biosecurity (Experiment 6) and this technique could also be used as a means of reducing toxic glycoalkaloids from raw potato waste (Experiment 5). Using the technique of lactic acid fermentation to provide biosecurity in liquid pig diets is a very new technology (Urlings *et al.* 1993). It was demonstrated that a greater degree of biosecurity could be achieved in diets containing liquid food industry residues (Experiment 7). However, because some of these products are known to contain different populations of microorganisms it may be necessary to treat

them prior to inclusion in liquid diets for weaner pigs. Liquid food industry residues which cause particular problems may require sterilisation, possibly by chemical means (Varekamp 1996).

In order to gain control of fermentations in practical situations it may be necessary to consider insulating liquid feed tanks, and adding heat to the system during winter months especially where inoculants are being added to liquid diets. It may be important in future to combine this with the use of an organic acid to optimise the growth rates of inoculants.

Within this study it was only possible to examine a few inoculants. However, in future it will be important to evaluate many more species of lactic acid bacteria in order to match the correct inoculant with the substrate of the diet. It is also possible to identify lactic acid bacterial inoculants which produce bactericidal agents which have specific antagonistic affects against spoilage and pathogenic organisms which may be present in liquid food industry residues. This approach could be applied to complete liquid diets or to individual liquid food industry residues. The potential for this application has already been demonstrated. For example, *Lactobacillus delbrueckii* subsp. *lactis* was found to be inhibitory to test strains of *Listeria monocytogenes* in milk (Tharrington *et al.* 1992); strains of *Enterococcus faecium* 7C5 strongly inhibited *Listeria innocua* (Tarelli *et al.* 1994); strains of *Pediococcus pentosaceus*, *Pediococcus acidilactici* and *Lactobacillus acidilactici* were found to be inhibitory against *Yersinia enterocolitica* in cooked meats (swine have been implicated as a major reservoir of *Yersinia enterocolitica* serotypes involved in human infections) (Raccach and Henningsen 1984). If this technology was applied to specific liquid food industry residues to create a product which was bio-secure, and specific against pathogenic organisms which have the potential to transmit food poisoning microorganisms then this could make a significant contribution to quality meat production.

Fermentation both changes the composition of the diet, for example by converting complex carbohydrates to simple sugars, and results in some loss of gross energy through carbon dioxide. Paradoxically, fermentation may increase the digestible energy or metabolisable energy content of the diet (by producing more readily digestible energy sources) while reducing the gross energy of the diet. This has two consequences. First, nutritionists may find it necessary to adjust nutrient specifications when formulating diets in order that a correct nutrient balance is still achieved after fermentation. Secondly, it may be necessary to apply correction factors to conventional proximate analyses to account for volatile fractions which may be lost during the analytical procedures.

It is normal practice when evaluating dietary components of animal feed to use proximate analysis techniques (James 1995). However, in fermented liquid diets there are several factors which will influence the way in which the data can be interpreted. For example, alcohol was present in the liquid diets in Experiment 4 at approximately 2 - 3%, and occasionally at 4.8%. When liquid feed samples are heated during the process of proximate analysis the volatile fractions (alcohol and volatile fatty acids) will evaporate and, therefore, will not be accounted for when calculating the digestible energy of the diet, using the equation of Whittemore (Equation No. 8.22, Whittemore 1993). This may partially explain why the energy level of the fermented liquid diet used in Experiment 4 (Treatment **LA**) was reduced by 0.91 MJ DE in the first week of fermentation. It was also demonstrated that fermentation of food industry liquid residue diets (Experiment 7) resulted in a considerable loss of moisture and sometimes dry matter. In order to account for this loss the data was corrected using ash as the mineral content of the diet would remain constant. In future it may be more efficient to consider adding chemically inert markers to the diet which can then be used as correction factors in future experiments of this kind.

In conclusion these studies have demonstrated that, the liquid feeding of weaner pigs can be successful providing the following criteria are applied:

1 The liquid diet is allowed to ferment (preferably with the addition of a lactic acid bacterial inoculant) until the pH is reduced to at least pH 4.2, thereby eliminating most potential pathogens from the diet. This would normally be achieved in order to mimic the feeding pattern that the pig has been receiving while suckling;

2 The liquid diet is fed *ad libitum*;

3 The diet is consistent, well balanced, highly digestible and provided in a liquid form containing not less than 220 g kg^{-1} of dry matter;

4 Piglets are weaned at approximately 24 ± 2 days and weighing in excess of 7 kg;

5 The correct environmental conditions are provided, *i.e.* sufficient pen and trough space, and a constant temperature (between 24°C and 26°C).

Given these conditions piglets could be expected to grow at up to 474 g d^{-1} between weaning (24 days) and 52 days and to have feed conversion ratio's as low as 1.15. This represents a significant improvement on the performance being achieved on most commercial farms and hence indicates an opportunity for producers to improve both productivity and profitability.

Table A 1. Calibration figures for effluent tanks

Pen	Litres per cm depth
1	17.20
2	17.35
3	17.43
4	17.60
5	17.68
6	17.84
7	17.59
8	17.35
9	17.36
10	17.74

Table A 2. Calibration figures for liquid feed dispensers

		Pen	Litres per dispensation
System	1	1	1.046
		3	0.954
		5	0.958
		7	0.937
		9	0.949
System	2	2	0.949
		4	0.959
		6	0.937
		8	0.953
		10	0.954

Table A 3. Relationship between the specific gravity and the proportion of ethanol in alcohol solutions at 20°C.

Specific gravity	% ethanol w/v	Specific gravity	% ethanol w/v
1.0000	0.00	0.9800	12.65
0.9995	0.26	0.9795	13.02
0.9990	0.53	0.9790	13.40
0.9985	0.80	0.9785	13.78
0.9980	1.06	0.9780	14.17
0.9975	1.33	0.9775	14.55
0.9970	1.61	0.9770	14.93
0.9965	1.88	0.9965	15.32
0.9960	2.16	0.9760	15.70
0.9955	2.44	0.9755	16.09
0.9950	2.72	0.9750	16.47
0.9945	3.01	0.9745	16.86
0.9940	3.30	0.9740	17.24
0.9935	3.59	0.9735	17.63
0.9930	3.88	0.9730	18.01
0.9925	4.18	0.9725	18.39
0.9920	4.48	0.9720	18.77
0.9915	4.78	0.9715	19.15
0.9910	5.09	0.9710	19.53
0.9905	5.40	0.9705	19.90
0.9900	5.71	0.9700	20.28
0.9895	6.03	0.9695	20.66
0.9890	6.35	0.9690	21.03
0.9885	6.67	0.9685	21.40
0.9880	7.00	0.9680	21.77
0.9875	7.33	0.9675	22.13
0.9870	7.67	0.9670	22.49
0.9865	8.00	0.9665	22.85
0.9860	8.34	0.9660	23.21
0.9855	8.69	0.9655	23.57
0.9850	9.03	0.9650	23.92
0.9845	9.38	0.9645	24.27
0.9840	9.73	0.9640	24.61
0.9835	10.09	0.9635	24.95
0.9830	10.44	0.9630	25.29
0.9825	10.80	0.9625	25.63
0.9820	11.17	0.9620	25.96
0.9815	11.53	0.9615	26.29
0.9810	11.91	0.9610	26.61
0.9805	12.28	0.9605	26.93
0.9800	12.65	0.9600	27.25

APPENDICES

Table A 4. Weaning record sheet (example for one treatment)

Date of Weaning .....
Treatment .....

Gilts				Boars		
Identity	Weight	Age	Pen	Identity	Weight	Age
			2			
			4			
			6			
			8			
			2			
			4			
			6			
			8			
			2			
			4			
			6			
			8			

Weight range .....
Age range .....

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